A study of the neonatal haematology of children with Down syndrome

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Abstract

This thesis describes the establishment and initial findings of the Children with Down Syndrome Study, a birth cohort of children with DS. The Children with Down Syndrome Study was set up in order to characterise the haematology of neonates with Down syndrome and specifically to test the hypothesis that that this differed in this population. The study was carried out with the support of the Down Syndrome Association and the Down Syndrome Medical Interest Group, and through consultation with clinicians and families. Following a pilot study in the Yorkshire region it was established in over 60 hospitals across the north of England.

The Children with Down Syndrome Study is the largest birth cohort of children with Down syndrome established to date, and this is the largest reported analysis of the haematology of neonates with Down syndrome. The results confirm that neonates with Down syndrome have a distinct haematological profile. Means and ranges for haematological parameters throughout the neonatal period are provided. The effects of gestational age, birth weight, postnatal age and the venepuncture to processing interval on the neonatal full blood count were examined, and this is the first report of factors that influence the haematological parameters in neonates with Down syndrome.

In order to analyse the blood cell morphology a new approach to morphology was developed and validated. Morphological review of samples from neonates with Down syndrome demonstrated that blasts were common. Comparison with automated findings showed that manual review of a film is indicated to look for evidence of transient myeloproliferative disorder.

This is also the largest longitudinal study of haematological parameters of children with Down syndrome beyond the neonatal period. The results showed that parameters changed between birth and 1 year, with most being stable thereafter. However, it appears that children with Down syndrome continue to have a distinct morphological profile.
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Author’s declaration

This candidate confirms that the work submitted is her own, and that where appropriate credit has been given to the work of others.
Chapter 1 Introduction

1.1 Purpose

The first recognizable description of Down syndrome (DS) occurs in 1886 in a case report by John Langdon Down who recognised phenotypic similarities within a subset of institutionalised adults (Down, 1866). Over the subsequent years features seen as part of the DS phenotype were increasingly well defined, although at the same time it became evident that there was very marked heterogeneity within DS. More than eighty years after the report by Langdon Down, LeJeune described the causal association between a third chromosome 21 and the DS phenotype (LeJeune, Turpin and Gautier, 1959), which results from non-disjunction in ~95%; translocation in ~4%; or chromosomal mosaicism in ~ 1% (Nicolaidis and Petersen, 1998).

Importantly, although children with DS may have myriad health problems, medical understanding - and consequently management - of these is based on a surprisingly small evidence base. It is well recognised that children with DS are at increased risk of developing serious haematological disease such as acute leukaemia (Krivit and Good, 1957; Hasle, Clemmensen and Mikkelsen, 2000; Yang, Rasmussen and Friedman, 2002; Hill et al, 2003; Agarwal et al, 2005), and children with DS are therefore over-represented in paediatric haematology wards and clinics. However, more subtle abnormalities of the full blood count are also fairly frequent, and yet there is little published data on these, making it difficult to respond to requests for advice on management with any degree of confidence.

The central hypothesis underpinning this work is the supposition that the haematological profile of neonates with DS is distinct and that if studied systematically in as near a population based cohort as possible then it will be possible to characterise this profile. Further, it may then be possible to determine variables affecting the haematology of this specific population.

As well as the numerical parameters obtained from automated analysis, information about blood cells can be provided by morphological analysis of a blood film. If the numerical parameters are distinct in DS then it is likely that the morphological appearances in DS may also be distinct. Moreover, morphological analysis may yield additional information which is important clinically.
1.2 Aims

The primary aims of this work are:

1. To describe the neonatal haematological ranges for children with DS with reference to the standard haematological parameters typically measured as part of full blood count analysis;
2. To examine factors that may be associated with variations in the neonatal haematological profile of children with DS;
3. To describe the features of neonatal blood cell morphology in children with DS;
4. To describe the haematological parameters in children with DS beyond the neonatal stage.

The secondary aims, which are related to but not directly part of this thesis, are:

1. To set up a study of children with DS which is capable of collecting information and biological samples from birth onwards;
2. To set the study up so that information about all aspects of health is collected and so that other studies may be nested within it;
3. To encourage collaborations with experts in different areas in order to maximise the potential of the study to investigate other areas of importance relevant to DS.

The Children with Down Syndrome Study (CDSS; www.cdss.org.uk) was set up specifically to address these aims. The CDSS is a collaborative project involving clinicians, scientists, families and other interested groups following a cohort of children with Down syndrome from birth onwards in order to develop a resource from which a robust evidence base might be developed to enable clinicians, and indeed the whole multi-disciplinary team, to provide the best care possible for children with DS – in the field of haematology and across all aspects of health.
Chapter 2 Critical assessment of the literature

2.1 Introduction

This chapter reviews the medical literature on neonatal haematology, considering first DS neonates, and then the wider population of non-DS neonates. Literature searches were conducted using PubMed search engines (www.ncbi.nlm.nih.gov/pubmed), citation searches, cross-referencing of papers and standard clinical text books.

To find relevant literature on the haematology of DS neonates the following terms were used in a PubMed search engine:

- haematology AND Down syndrome;
- acute leukaemia AND Down syndrome;
- acute myeloid leukaemia AND Down syndrome;
- acute lymphoblastic leukaemia AND Down Syndrome;
- transient myeloproliferative disorder AND Down syndrome;
- blood film AND Down syndrome;
- blood cell morphology AND Down syndrome.

In addition from the start of the research period weekly reports from PubMed of any paper with Down syndrome in the abstract, title or as a keyword were reviewed.

To find relevant literature on the haematology of the wider neonatal population the following terms were used in a PubMed search engine:

- reference range AND white cell count;
- reference range AND platelet;
- reference range AND red cell count;
- erythrocyte indices;
- blood cell morphology AND microscopy;
- platelet morphology AND microscopy.

Again, during the research period weekly reports from PubMed of any paper with any of the above terms in the abstract, title or as a keyword were reviewed.
2.2 The haematology of neonates with Down syndrome

Children with DS are susceptible to a spectrum of haematological abnormalities ranging from asymptomatic changes of the full blood count to an increased incidence of acute leukaemia – changes which may even be apparent in utero.

2.2.1 Benign haematological changes

The published reports of haematological indices in neonates with DS are shown in Table 2-1, and of children with DS beyond the neonatal stage in Table 2-2. It can be seen that much of the literature is based on anecdotal case reports or small series and it is perhaps surprising that only one prospective study of the haematology of DS neonates has been reported, and this is small – only including 25 neonates (Kivivuori, Rajantie and Siimes, 1996). Similarly, although the need for DS specific haematological reference ranges has long been recognised (Starc, 1992; Kivivuori, Rajantie and Siimes, 1996), none have been reported.
Table 2-1. Published reports of haematological indices in neonates with Down syndrome.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age range</th>
<th>Number of cases with DS</th>
<th>Type of study</th>
<th>Parameters studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behrman et al.,</td>
<td>Neonates</td>
<td>3 sibs (all with DS)</td>
<td>Case report; One followed up for 3 years</td>
<td>HB, HCT, MCHC, WBC, PLT</td>
</tr>
<tr>
<td>1966</td>
<td>Reports of 2</td>
<td></td>
<td></td>
<td>Blood cell morphology</td>
</tr>
<tr>
<td>Lappalainen et al,</td>
<td>Neonates</td>
<td>874 referred for medical treatment</td>
<td>Retrospective review</td>
<td>HB, HCT, Prothrombin level</td>
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<tr>
<td>1972</td>
<td>14 had DS</td>
<td>17 extra DS cases added</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kohne et al,</td>
<td>Neonates</td>
<td>11</td>
<td>Prospective Cross-sectional 6 month follow up in 3 cases</td>
<td>HCT, HbA1, HbA2, HbF</td>
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<tr>
<td>1975</td>
<td></td>
<td></td>
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<tr>
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<td>70</td>
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<td>1979</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miller et al,</td>
<td>Neonates</td>
<td>61 counts available in 51</td>
<td>Retrospective review</td>
<td>HCT, WBC, PLT</td>
</tr>
<tr>
<td>1983</td>
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<td></td>
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<td>Widness et al,</td>
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<td></td>
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<td>Hord et al,</td>
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<td>Cross-sectional</td>
<td>PLT</td>
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<td>Prospective</td>
<td>HB, HCT, MCV, WBC, PLT</td>
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<td>1996</td>
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<td>1 year follow up</td>
<td></td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>Henry et al,</td>
<td>Neonates</td>
<td>266 Results for 158</td>
<td>Retrospective</td>
<td>HB, HCT, NRBC, WBC, neutrophils, PLT</td>
</tr>
<tr>
<td>2006</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Table 2-2. Published reports of haematological indices in children with Down syndrome.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age range</th>
<th>Number of cases with DS</th>
<th>Type of study</th>
<th>Parameters studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ridler et al., 1959</td>
<td>2-15 months</td>
<td>8</td>
<td>Longitudinal</td>
<td>WBC</td>
</tr>
<tr>
<td>Roizen et al., 1983</td>
<td>2-6 years</td>
<td>18</td>
<td>Prospective, Cross-sectional 2 year follow up</td>
<td>HCT, MCV, thyroid function serum folate, red cell folate WBC in 14/18 with DS HB electrophoresis in 3/18 with DS reticulocytes in 2/18 with DS</td>
</tr>
<tr>
<td>Akin et al., 1988</td>
<td>Children</td>
<td>7</td>
<td>Not specified</td>
<td>HCT, HC, MCV, WBC</td>
</tr>
<tr>
<td>Starc et al., 1991</td>
<td>3 months – 7 years</td>
<td>63</td>
<td>Retrospective review</td>
<td>HB, HCT, RCC, MCV, MCH, MCHC</td>
</tr>
<tr>
<td>Bartelik et al., 1995</td>
<td>3 months - 10 years</td>
<td>62</td>
<td>Cross-sectional</td>
<td>MCV, HCT, serum iron, ferritin WBC</td>
</tr>
<tr>
<td>David et al., 1996</td>
<td>2-15 years</td>
<td>50</td>
<td>Cross-sectional</td>
<td>HB, HCT, RCC, MCH, MCHC, RDW, MCV, WBC, PLT, MPV, serum folate, red cell folate, vitamin B12 haptoglobin, red cell creatine hexokinase in 40 with DS</td>
</tr>
<tr>
<td>Dixon et al., 2010</td>
<td>1-19.7 years</td>
<td>114</td>
<td>Cross-sectional</td>
<td>HB, MCH, MCHC, MCV, RDW, TS, ferritin, FEP, ZCP</td>
</tr>
<tr>
<td>Bloemers et al., 2010</td>
<td>Children</td>
<td>41</td>
<td>Prospective</td>
<td>WBC and differential WBC count</td>
</tr>
</tbody>
</table>
The first reported abnormality of the full blood count in otherwise healthy children with DS was published in 1959 (Ridler and Shapiro, 1959). In this study eight infants with DS were reported to have lower mean white blood cell counts (WBC) than non-DS infants, although the difference was not statistically significant, and review of the medical records did not reveal frequent or unusual infections in the DS group. However, the numbers were small, follow-up was limited and there was no clear explanation of criteria used to identify or code infectious episodes. Two subsequent studies also found low WBCs in children with DS, but again the numbers studied were small and, although clinical information was provided in one study, this was minimal (Roizen and Amarose, 1993; Kivivuori, Rajantie and Siimes, 1996). More recently, a study of 41 DS children with age-matched controls has extended our understanding by demonstrating significantly lower absolute total leukocyte counts, lymphocytes, monocytes, and granulocytes, but 1.5-times higher numbers of pro-inflammatory monocytes in DS children (Bloemers et al, 2010a). The authors suggest that these monocytes might be involved in the development and maintenance of chronic inflammatory diseases often associated with DS (Scherberich, 2003).

One of the earliest papers on DS and haematology is a case report of three siblings with DS, two of whom had marked haematological changes as neonates including haemolytic anaemia, thrombocytopenia and myeloid metaplasia (Behrman, Sigler and Patchefsky, 1966). Thrombocytopenia has since been consistently demonstrated in DS neonates (Miller and Cosgriff, 1983; Thüring and Tönz, 1979; Hohlfeld et al, 1994; Hord, Gay and Whitlock, 1995; Kivivuori, Rajantie and Siimes, 1996; Henry et al, 2007). Thrombocytopenia has also been demonstrated in other trisomies. In a study of 86 neonates with a trisomy, 70 of which had DS, 60% had thrombocytopenia (Thüring and Tönz, 1979). The mean platelet count was 104.6 (45, 175) x 10⁹/l (mean; 10 and 90 percentiles) for those with DS. In the two studies with follow-up the thrombocytopenia resolved (Miller and Cosgriff, 1983; Kivivuori, Rajantie and Siimes, 1996) – and in fact there was subsequently a tendency to thrombocytosis in later infancy (Kivivuori, Rajantie and Siimes, 1996). This latter finding is not entirely surprising as thrombocytosis has also been demonstrated in infants without DS (Addiego, Mentzer and Dallman, 1974; Lundström, 1979; Heath and Pearson, 1989). This probably reflects the fact that healthy children are unlikely to have a blood test performed on them, and so full blood count data comes from children who are more likely to be compromised in some way, particularly as a result of acute infection. Thrombocytosis is a healthy physiological response to infection and so is not an unexpected finding in this context.

The first indication of the presence of red cell abnormalities in children with DS came from a study ascertaining neonates with polycythaemia from a general population of neonates, which
reported a 20-fold excess of DS amongst those with polycythaemia (Weinberger and Oleinick, 1970). Subsequently, a retrospective review of haemoglobin and haematocrit in babies referred for hospital treatment also found a raised haematocrit in the subgroup with DS (Lappalainen and Kouvalainen, 1972). This did not appear to be associated with concurrent cardiac disease – most of the congenital cardiac disease in DS is not cyanotic, and even with congenital cyanotic cardiac disease polycythaemia would not be expected to appear for several days. The authors suggested that placental dysfunction might be implicated. In response to these findings, a retrospective review of DS neonates was conducted, and confirmed that the haematocrit and platelet counts were significantly elevated and reduced respectively in DS neonates (Miller and Cosgriff, 1983). Another study measured erythropoietin levels, as a proxy marker of fetal hypoxaemia, in the umbilical cord blood of neonates with and without DS (Widness et al, 1994). The erythropoietin levels were significantly higher in the DS group, but again within this group no significant effect of concurrent cardiac disease was observed. Unfortunately, in this study the haematocrit was only available in a small number of cases. Interestingly, babies with DS were noted to have lower birth weights which could reflect fetal hypoxaemia resulting from placental insufficiency.

To date only one prospective study of full blood counts in neonates with DS has been reported, and this only followed 25 neonates for 12 months (Kivivuori, Rajantie and Siimes, 1996). Neonates known to have a haematological abnormality were excluded. Full blood counts were taken at frequent intervals, and although 64% infants were polycythemic in the first week of life, this resolved after 8 weeks. The pattern of change of haemoglobinaemia was noted to be similar to that already described in infants without DS, falling to a nadir at around 2 months and then rising slowly to reach a plateau towards the end of the first year of life.

The next red cell change to be described in children with DS was macrocytosis; this had been recognised in adults with DS since the late 1960’s (Naiman, Oski and Mellman, 1965; Eastham and Jancar, 1970; Howell et al, 1973; Akin, 1988; Wachtel and Pueschel, 1991). A retrospective review compared full blood counts from children with DS to those from two control groups: one with and one without cardiac disease (Starc, 1992). Both the mean red cell volume (MCV) and mean red cell haemoglobin (MCH) were significantly higher in children with DS. Additionally, the haemoglobin and haematocrit were higher in DS, but this was only significant in comparison to the control group without cardiac disease. Interestingly, for DS children having concurrent cardiac disease made little difference until 2-7 years of age when, compared to those without cardiac disease, a significantly higher haemoglobin, MCH and mean cell haemoglobin concentration (MCHC) was observed. The authors of this review make the point that comparing the full blood count from a child with DS against standard
parameters might be misleading, and state the case for establishing reference values for haematology parameters in DS. For example, a child with DS and iron deficiency might have an MCV that was low for DS, but which appeared normal when compared to standard charts. In this scenario, iron deficiency might not be diagnosed until late in its course. This possibility has been investigated in a study of 114 children with DS, which found iron deficiency/iron deficiency anaemia was present in 15 children. However, it would have been missed in 13 of these 15 children if red blood cell indices alone, and not iron studies, had been used for screening (Dixon et al, 2010).

The association between red blood cell macrocytosis and DS was confirmed in a retrospective analysis of 60,000 full blood counts of which 146 were found to be macrocytic (Pappo, Fields and Buchanan, 1992). The most common association was with drugs, but there was an excess of children with DS amongst those with macrocytosis. Following on from this came the first report of full blood counts from prospectively recruited healthy children with DS who were not institutionalized (Roizen and Amarose, 1993). Importantly, this study included a control group matched for age and sex. Both the MCV and the haematocrit were significantly higher in DS. There was no significant difference in red cell or serum folate levels, and although compensated hypothyroidism was not uncommon in the DS group it did not appear to be associated with a high MCV. Three cases were also examined by haemoglobin electrophoresis, but there was no sign of persisting fetal haemoglobin. Reticulocytes were looked for in two samples, but were absent. It had previously been speculated that the high MCV might be an early sign of bone marrow failure, but there was no report of associated bone marrow failure in over two years of follow up.

Another study of full blood counts in DS also looked at a range of relevant red blood cell parameters in 50 children with DS and 68 age matched controls in an attempt to determine the underlying cause of the macrocytosis (David et al, 1996). In addition to a significantly higher MCV, it found a significantly higher haemoglobin, MCH, haematocrit, and red cell distribution width. However, red blood cell count, MCHC, red cell and serum folate, vitamin B12, haptoglobin, red cell creatine, serum iron, ferritin and hexokinase were not significantly different from non-DS controls. The authors postulate that the high MCV might reflect enhanced erythropoiesis in response to impaired oxidative metabolism, and further propose that this might occur as a gene dosage effect given that the gene for a superoxide dismutase (SOD1) is located on chromosome 21 - superoxide dismutases are essential enzymes that eliminate superoxide radicals and thus protect cells from damage induced by free radicals. Alternatively the authors consider whether there might be an alteration in the folate remethylation pathway resulting from reduced thymidylate synthase activity and consequent
prolonged RNA synthesis in the red cell precursor - the gene for cystathione β synthase, regulating a critical step of the re-methylation pathway, is found on chromosome 21. This latter theory is compatible with normal folate parameters.

The only longitudinal data available on neonates with DS (Kivivuori, Rajantie and Siimes, 1996) not only confirmed red cell macrocytosis, but also showed an increase in MCV over the first year of life. This was not observed to be associated with a reticulocytosis and serial erythropoietin levels were low to normal. In view of the multiple potential haematological abnormalities the authors iterated the need for reference values for full blood counts in DS infants to help management.

The largest report of full blood counts in neonates with DS was published recently – one of a series of reports on haematological parameters in neonates to have come from the Intermountain Healthcare Hospital organization which represents 23 hospitals in Salt Lake City, Utah and in the surrounding area (Henry et al, 2007). In this retrospective review of computerised medical records 266 neonates with DS were identified. Of these, 158 had had full blood counts taken in the first week of life. The most common finding was of neutrophilia, present in 80%. Thrombocytopenia and polycythaemia were noted, consistent with previous reports, and further analysis showed a correlation between the two, with polycythaemia being almost three times as likely if thrombocytopenia was present. Blasts consistent with a diagnosis of transient myeloproliferative disorder were noted in 6%. In contrast, the incidence of anaemia, thrombocytosis and neutropenia appeared similar to the general population.

There were, however, several problems with this study: not all DS neonates had a full blood count raising the possibility that those who did had adverse clinical features; clinical information was limited; there no information about the source of sample – an important variable; and finally, there was no information about the morphology.

Taken together it is clear that various abnormalities of the full blood count are associated with DS at the neonatal stage. These may include polycythaemia, macrocytosis, thrombocytopenia, leucopenia and neutrophilia. However, the quality of much of the data is limited, most being based on anecdotal reports or on small series. One possible reason for the paucity of data is that the haematological changes have not usually been associated with serious clinical disease. Moreover, although follow-up are limited, it has been suggested that most haematological abnormalities tend to resolve spontaneously over the first year of life – indeed this is the standard teaching in medical textbooks. However, there is now increasing evidence that some haematological changes present in DS neonates might actually be very
significant in predicting a group at particularly high risk of early serious haematological disease, specifically myeloid leukaemia of Down syndrome (ML-DS).

### 2.2.2 Malignant haematological disorders

The first case report of acute leukaemia in DS was published in 1930 (Brewster and Cannon, 1930). Subsequently, a postal survey by Krivit and Good confirmed a growing suspicion that acute leukaemia occurred with increased frequency in children with DS (Krivit and Good, 1957). Large epidemiological studies from the 1990s onwards have consistently reported a 20-30 fold increased risk of acute leukaemia in DS (Hasle, Clemmensen and Mikkelsen, 2000; Yang, Rasmussen and Friedman, 2002; Hill et al, 2003; Agarwal et al, 2005). Interestingly, this increase is not mirrored by other cancers. Indeed, the available epidemiological evidence suggests that while the incidence of testicular cancer may be increased, the incidence of many other cancers appears to be reduced (Bennett et al, 1976; Hasle, Clemmensen and Mikkelsen, 2000; Hill et al, 2003; Goldacre et al, 2004).

Much of the data on leukaemia in DS is derived from trial series, which may be incomplete and potentially subject to bias. Trial entry for children with DS has lagged behind that of the general population. Indeed, data from the UK Childhood Cancer Study, a national population-based study which recorded all diagnoses of childhood cancer and leukaemia over a four year period from 1990-1994, indicated that while trial entry approached 90% in non-DS children with leukaemia, it was greatly reduced in those with DS being around two-thirds for those with acute lymphoblastic leukaemia (ALL) and half for those with acute myeloid leukaemia (AML) (James et al, 2008). Until trial entry improves, population based studies remain a more robust way of examining this high risk population.

**Transient myeloproliferative disorder**

Transient myeloproliferative disorder (TMD; also referred to as transient abnormal myelopoiesis; or transient leukaemia) is a clonal myeloproliferative disorder of myeloid origin which has been recognised in neonates with DS for over 50 years (Schunk and Lehman, 1954). Typically the child is asymptomatic and the disorder resolves spontaneously within 3 months (Zipursky and Doyle, 1993; Zipursky et al, 1997; Zipursky, 2003). In a small minority of patients there are clinical changes, notably liver fibrosis, pleural and pericardial effusions and disseminated intravascular coagulation. When these occur the child may be very sick, and without treatment they are life threatening. The exact incidence of TMD is unknown – historically the diagnosis was not routinely sought and there are no agreed diagnostic criteria. Typically, there is a high WBC count, with blasts on the blood film often with
thrombocytopenia, a leukoerythroblastic picture and megakaryocyte fragments (Zipursky et al, 1999). The oft-quoted incidence of 10% is derived from a study by Zipursky which reported an incidence of 10% in a small series that examined blood films from newborn DS infants (Doyle and Zipursky, 1994). Another study examined 79 DS fetuses by ultrasound for hydrops fetalis and hepatosplenomegaly, using these as proxies for TMD, and reported an in utero prevalence of 14% (Smrcek et al, 2001). Zipursky has suggested that the population picked up by ultrasound in utero are at high risk of intrauterine death, and that those picked up postnatally are an additional group, giving a total prevalence of TMD in utero of approximately 20% and in newborns of approximately 10% (Zipursky, 2003). However, it is not clear whether hydrops fetalis and hepatosplenomegaly can always be assumed to equate to a diagnosis of TMD in a fetus with DS. There are certainly many other causes of hydrops fetalis (White, 1999), and it has been described in DS in association with other diagnoses (Fujimoto et al, 1983; Ilagan et al, 1992; Rutigliani et al, 2007). Moreover, in contrast to the higher estimates from older studies, two recent studies both reported much lower frequencies (Ahmed et al, 2004; Pine et al, 2007). TMD was only diagnosed in 9/158 (6%) full blood counts taken from DS neonates when these were analysed retrospectively (Henry et al, 2007). Another retrospective analysis, this time of Guthrie cards of children recorded as having DS on a congenital malformation registry, found a GATA1 mutation and inferred a diagnosis of TMD in 22/590 (4%) cases (Pine et al, 2007).

The significance of TMD has been unclear until recent years, and as most children are well and as the changes typically resolve spontaneously, it has previously attracted little clinical attention. Similarities between acute megakaryoblastic leukaemia (AMKL) and TMD have long been recognised, for example, the blasts have a similar immunophenotypic profile (Yumura-Yagi et al, 1992; Ito et al, 1995; Litz et al, 1995; Karandikar et al, 2001; Langebrake, Creutzig and Reinhardt, 2005). A significant breakthrough came in 2002 when a consistent mutation of GATA1, producing a short form of GATA1 (GATA1s), was demonstrated exclusively in children with AMKL and DS – but not in children with AMKL without DS, in DS children with other types of acute leukaemia, or in healthy DS children (Wechsler et al, 2002). GATA1 is a key DNA-binding transcription factor that regulates the growth and maturation of erythroid cells, megakaryocytes, eosinophils and basophils/mast cells. It is not expressed in lymphocyte or other myeloid lineages. It is encoded on the X chromosome and has a phosphorylated double zinc finger structure. Both zinc fingers help GATA1 bind DNA and interact with other proteins. Other groups have since confirmed that a GATA1 mutation is pathognomonic of AMKL in DS and have demonstrated similar mutations of GATA1 in DS neonates with TMD (Hitzler et al, 2003; Mundschat et al, 2003; Rainis et al, 2003; Xu et al,
Follow up of several individual cases has shown that the mutation becomes undetectable as the TMD resolves, but may recur with the development of AMKL (Shimada et al, 2004; Xu et al, 2006; Kanegane et al, 2007). Examination of Guthrie cards for children with DS diagnosed with AMKL in early childhood revealed similar GATA1 mutations (Ahmed et al, 2004). There are few prospective studies of neonates with TMD. A prospective follow up study of 48 newborns diagnosed with both DS and TMD reported the subsequent development of AMKL in 8 (17%) and ALL in 1 (2%) (Massey et al, 2006). The same group have recently reported on a larger group of 135 DS neonates with TMD of whom 21 (16%) developed MDS or AML with a median time of 441 days (Gamis et al, 2011). Interestingly, 4 of the 21 who developed MDS or AML had received intervention therapy for TMD, typically with intravenous cytarabine. A further 2/135 neonates developed ALL. A European report of 146 infants with TMD found a similar incidence of subsequent TMD with 23% developing myeloid leukaemia after 5 years of follow up (Klusmann et al, 2008). A retrospective Japanese study of 70 infants with TMD reported an increase in early death in those with a white cell count of >100 x10⁹/l and for preterm infants, but did not look at subsequent malignancy (Muramatsu et al, 2008). It is not possible at present to predict which neonates with TMD are likely to develop subsequent leukaemia, although significant correlations with the later development of AML and karyotypic abnormalities in addition to trisomy 21 (Massey et al, 2006) and also with an increased time taken for the TMD to resolve (Gamis et al, 2011) have been described.

One of the problems in this area has been a lack of agreed diagnostic criteria for TMD. This reflects the initial uncertainty as to whether it was or was not a malignancy – an uncertainty which arose from its unusual clinical features, particularly the typical wellness of the child and the spontaneous regression. In the most recent study of children with TMD (Gamis et al, 2011) it was defined as follows: ‘<3 months of age at presentation with any non-erythroid blasts in the peripheral blood coupled with any of the five following criteria: verification of blasts with a second sample, >5% non-erythroid bone marrow blasts, hepatomegaly or splenomegaly, lymphadenopathy, or cardiac or pleural effusions…organomegaly or adenopathy were determined by the clinician’s physical exam and did not have strict criteria’.

Given the association of GATA1 and TMD it may well be that assessment of GATA1 status forms part of new diagnostic criteria for TMD, but it is important to note that this has not been the case in the majority of literature to date and it is not currently in routine clinical use.
Acute myeloid leukaemia

Both myeloid leukaemia of Down syndrome (ML-DS), a specific disease within this population; and sporadic AML, representing a heterogeneous group of AML subtypes, appear to be increased in DS.

Myeloid leukaemia of Down syndrome

AMKL occurs ~500 fold more often in children with DS compared with the general paediatric population (Zipursky, Poon and Doyle, 1992; Lange et al, 1998; Creutzig et al, 1996; Khan, Malinge and Crispino, 2011). It is now clear that GATA1 mutations, resulting in GATA1s, are acquired in utero, and are associated with TMD. Typically, this will resolve, but the GATA1 may reappear in association with the development of AMKL (Gurbuxani, Vyas and Crispino, 2004). There are several unusual features of AMKL in DS: it occurs in the first 5 years of life; tends to be preceded by a myelodysplastic prodrome of 4-24 months (Creutzig et al, 1996); and is exquisitely sensitive to chemotherapy (Zwaan et al, 2000). Additionally, there is a suggestion that response to treatment is improved further if it is treated early (Creutzig et al, 2004). Cytogenetic analysis typically shows absence of favourable chromosomal translocations such as t(8;21), t(15;17), inv 16, t(1;22), although unbalanced translocations, particularly +8, del 6q, +11, +21 appear to be increased (James et al, 2008).

Given that the natural history of myelodysplasia in DS is progression to AMKL, and as there is no biological, prognostic or therapeutic reason to differentiate them, the term myeloid leukaemia of DS (ML-DS) was proposed to encompass myelodysplasia and AMKL with an obligate GATA-1 mutation in children with DS (Hasle et al, 2003).

Sporadic AML

Myeloid leukaemia in children 4 years or older with Down syndrome often lacks a GATA1 mutation, whilst the cytogenetic changes and risk of relapse are more akin to sporadic AML. Analysis of AML subtypes by several groups has suggested that acute erythrocytic leukaemia may be specifically increased in children with DS (Levitt, Stiller and Chessells, 1990; Creutzig et al, 1996; Lange et al, 1998; James et al, 2008), whilst other studies have shown a spread of subtypes (Agarwal et al, 2005; Abella et al, 2008).

Acute lymphoblastic leukaemia

The discovery of an exclusive mutation of GATA1 in children with DS and AMKL has generated considerable excitement in the paediatric haematology world. However, the attention concentrated on this has somewhat obscured the fact that in absolute terms acute
lymphoblastic leukaemia (ALL) remains at least as common as AML in children with DS (Rosner and Lee, 1972; Hill et al, 2003; Agarwal et al, 2005; James et al, 2008). The mean age at diagnosis for DS-ALL is 4.8 years, which is not significantly different to non-DS ALL (James et al, 2008). ALL in DS is a more heterogeneous disease than AML in DS, which is a distinct entity, and there are distinct clinical and biological features which distinguish it from non-DS ALL (Maloney, 2011). Precursor B-cell ALL is the predominant form, with T-cell ALL being extremely rare (Pui et al, 1993; Chessells et al, 2001; Bassal et al, 2005; James et al, 2008; Maloney et al, 2010). High hyperdiploidy is typically underrepresented, and chromosomal translocations associated with adverse outcomes such as 11q23/MLL, t(9;22), t(1;19) are often absent (Pui et al, 1993; James et al, 2008; Maloney et al, 2010). An activating mutation of JAK2 has recently been demonstrated in ~20% DS, compared with ~10% non-DS high risk ALL, and appears to be associated with a distinct ALL phenotype (Bercovich et al, 2008).

### 2.2.3 Blood cell morphology

Most of the papers that report any blood cell morphology in neonates with DS have done so in the context of TMD. There is one report of blood cell morphology in 13 young children with DS which noted a common finding of large platelets with abnormalities of the membrane and granules (Boisseau et al. 1976).

In the papers pertaining to TMD several morphological features, other than simply the presence of blasts, have been documented. There are often platelet abnormalities with thrombocytopenia (Cantù-Rajnoldi et al, 1988; Cook et al, 1989; Zipursky et al, 1999; Henry et al, 2007), and the presence of megakaryocytes or megakaryocyte fragments in the peripheral blood (Suda et al, 1988; Zipursky et al, 1999; Webb, Roberts and Vyas, 2007) being most commonly reported. Although the latter review also states that giant platelets are a feature, this is not substantiated by data (Webb, Roberts and Vyas, 2007). There is one mention of thrombocythaemia – in a report of one patient who also had a neutrophilia, eosinophilia and basophilia (Suda et al, 1987).

Zipursky, who pioneered the morphological and clinical advances in recognising and understanding TMD describes a leukoerythroblastic picture (Zipursky et al, 1999) and early reports described a leukaemoid reaction (Cantù-Rajnoldi et al, 1988; Fernandez de Castro et al, 1990) with some reports specifically commenting on the presence of nucleated red blood cells (Henry et al, 2007; Webb, Roberts and Vyas, 2007).
Where the morphology of the blasts is described in TMD they are reported to have the features of megakaryoblasts with blebbed cytoplasmic outline (Zipursky, Poon and Doyle, 1992) although a recent review discussing TMD states that there are ‘deeply basophilic blast cells usually indistinguishable from neonatal blasts but present in greater number’ (Webb, Roberts and Vyas, 2007). A small series of 4 children with DS and acute leukaemia includes 2 neonates (Brunning et al, 1974). This notes the presence of prominent basophilic inclusions in some, but not all, blasts. A study of 5 patients with TMD looked at the ultrastructural features of the blasts and concluded that they were heterogeneous with evidence of megakaryocytic, granulocytic and erythroid lineages (Bessho et al, 1988). This diversity calls into question the initial diagnosis of TMD in this paper, especially as Zipursky comments that blasts are frequent in neonates with DS whether or not they have TMD (Zipursky, Poon and Doyle, 1992), and highlights again the need for clear diagnostic criteria for TMD. Similarly, in the large retrospective review of the haematology of DS neonates blasts were noted in 9/158 cases, of which 3/9 were referred to a haematologist for follow up because of an increasing blasts count which was inferred to indicate TMD (Henry et al. 2007).

2.3 Existing and historical cohort studies

DS cohort studies are rare. There are only two comparable birth cohorts: the National Down Syndrome Project (NDSP), based in Atlanta (Freeman et al, 2007), and a prospective birth cohort (Bloemers et al, 2007) developed in Holland. The NDSP, which recruited children between 2001 and 2004, grew out of the Atlanta Down Syndrome Project, which had recruited children between 1989 and 1999. The NDSP was designed to examine the molecular epidemiology of DS and to assess risk factors for DS associated congenital defects. Although 907 infants were recruited, follow up ceased after a single telephone interview. The Dutch cohort study recruited 241 children with DS between 2003 and 2005, and has followed them until two years of age. To date, the Dutch cohort has reported on the risk of respiratory syncitial virus (Bloemers et al, 2007), recurrent wheeze (Bloemers et al, 2010b), immune dysregulation (Bloemers et al, 2010a; Bloemers et al, 2011) congenital heart defects (Weijerman et al, 2010) in children with DS. Neither the NDSP nor the Dutch cohort has addressed haematological issues.

2.3.1 Summary

The need to describe the haematology of DS neonates in a systematic manner and to establish reference ranges for neonates with DS is clear. In addition to determining these, it is important that clinical information is collected. There are many potential associations between neonatal haematological changes and important clinical conditions, including serious
contemporaneous conditions such as neonatal sepsis. Once the reference ranges are developed, it will be important to determine whether deviations from them have similar significance to deviations in non-DS neonates. There are also DS specific neonatal conditions, notably TMD, that are not fully understood. There is a clear discrepancy between older and more recent reports of the incidence of TMD, and a better understanding of the normal neonatal haematology of DS may help explain this. Similarly, there are unanswered questions about the neonatal management of DS neonates that will be addressed by this work, including the basic question of whether a FBC should be checked in all children with DS. Finally, looking towards the future, as the clinical outcome of children can be considered alongside the natural history of haematological indices it may be possible to highlight features that are of prognostic significance. All this will mark a very significant contribution to the healthcare of neonates with DS.

2.4 Haematological reference ranges for neonates

2.4.1 Introduction

This section considers the development of haematological reference values for non-DS neonates. The CDSS is an observational study of DS neonates. It would not have been appropriate to take samples from healthy non-DS neonates in order to construct contemporaneous reference ranges as these already exist. Moreover, it makes more sense to compare the values obtained in the CDSS with these existing ranges as this is what they will be compared with in daily clinical practice. However, it is very important that the development of the existing ranges is understood in order to understand their strengths and weaknesses. The term reference, and not normal, is used as blood samples are not usually taken from healthy neonates. This has not always been the case. It is apparent that reports from the first half of the last century do include results from healthy neonates, particularly those who were in socially vulnerable situations at a time when there were less stringent ethical constraints upon research. Adherence, rightly, to ethical considerations (Rice, 2008; Puri et al, 2009) means that although accurate automated techniques for determining haematological values are now well developed it is very difficult to study healthy children, as this would require an invasive procedure (Coleman, 2007).

The first reports of haematology parameters in neonates were published at the end of the 19th century: in 1866 von Korber identified that the haemoglobin of neonates, now recognised as fetal haemoglobin, was more resistant to alkalinisation than adult haemoglobin (von Korber, 1926); soon after this Schücking presented a formulae for calculating neonatal blood volume (Schücking, 1879). Although more recent reports have considered red cell, white cell and
platelet parameters on the same sample, older reports typically concentrated on one cell type – reflecting the limitations of older analysers and of manual counting. Accordingly, this discussion will focus on each cell type in turn. Today full blood count (FBC) analysis is an integral part of the management of sick neonates. However, before the 1970s the FBC was not thought to be especially useful in neonatology. Since then, and particularly as a result of the pioneering studies done by Rosenfeld's group in Dallas in determining reference ranges (Manroe et al, 1979; Mouzinho et al, 1994), the FBC has become of great importance in the management of sick or preterm neonates.

In the last few years an invaluable contribution to the field of neonatal reference ranges has been made by the Intermountain Healthcare Hospitals organization which represents 23 hospitals in Salt Lake City, Utah and in the surrounding area. This group have published reference ranges based on retrospective computerised results from very large data sets of neonates (Christensen et al, 2008; Schmutz et al, 2008; Christensen et al, 2009; Wiedmeier et al, 2009; Jopling et al, 2009; Christensen et al, 2010; Christensen et al, 2011). The electronic records store demographic and clinical information, including history and examination results, problem lists and discharge summaries as well as the computerised laboratory records required for analysis. The same automated counter, a Beckman Coulter LH750, was used in all the hospitals. There are, however, several weaknesses to these reports. Although clinical records are used to exclude neonates with features known to affect the parameters being studied some in these categories may have been included as records are not always complete. Furthermore, all reports are retrospective and lack important information about the source of the sample so that the ranges include samples from a variety of sources. Also, there is no information about morphological assessment. Taken together these weaknesses would tend to give wider reference ranges. A further problem is that the hospitals in this geographical area are at high altitude, which has been shown to affect red and white blood cell indices in neonates. However, despite these limitations this body of work provides the best reference sets to date, and as well as contributing to the clinical management of neonates, these provide an excellent and timely reference for the CDSS.

2.4.2 Measurement of haematological parameters

The first reports of haematological parameters relied on manual techniques involving simple reagents, centrifuges, glass slides, microscopes, counting chambers and colorimeters from which the haemoglobin concentration, packed cell volume (PCV) and red blood cell count (RCC) could be estimated. Further red cell indices, notably the mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) could then
be derived using simple calculations. These manual techniques were refined and used for many decades (Wintrobe, 1929a; Wintrobe, 1929b; Spivak, 2003) until they were superseded by automated blood cell analysers based on the principle of impedance counting able to measure indices directly (Coulter, 1956). Automated counting has since developed considerably, with newer machines incorporating a variety of techniques such as fluorescence flow cytometry, laser light scatter, white light scatter and absorbance, direct current (for impedance measurements) and radiofrequency current (for determining the internal structure of a cell). Modern analysers are precise, accurate and have the advantage of being able to turn over a large number of samples rapidly with little manual input. Manual techniques remain important in determining reference values and in investigating anomalous automated results (Barnes et al, 2005), but are no longer employed routinely. The established techniques used by modern analysers and the recommended units used to measure haematology parameters are described in Appendix 1.

Several organisations are involved in the oversight of the techniques used in haematology laboratory analysis. The International Society for Laboratory Hematology (ISLH; [www.islh.org](http://www.islh.org)) is a non-profit organisation with an international membership of laboratory professionals and is closely allied to the recently revived International Council for Standardisation in Hematology (McFadden et al, 2008). Both organisations are dedicated to the development of standards and guidelines for laboratory haematology to enable increasingly reliable and reproducible results. At a more local level, hospital haematology laboratories take part in a national accreditation process, administered by the United Kingdom Accreditation Service (UKAS; [www.ukas.com](http://www.ukas.com)) and Clinical Pathology Accreditation UK Ltd (CPA; [www.cpa-uk.co.uk](http://www.cpa-uk.co.uk)), and regular external quality assessment, co-ordinated by the UK National External Quality Assessment Scheme for Haematology (NEQAS; [www.ukneqas.org.uk](http://www.ukneqas.org.uk)). Further details are given in Appendices 2 and 3.
2.5 Red blood cell parameters in neonates

2.5.1 Development of reference ranges for red blood cell parameters

‘Many writers have noted the lack of standard values for the number of red corpuscles, amounts of haemoglobin and percentage of packed corpuscles in the blood of the newborn infant’

Quinn DeMarsh, Factors influencing the blood picture of the newborn, 1948

Several reports at the turn of the 19th century identified differences in neonatal blood parameters compared with adult blood – notably, a significantly higher HB and larger RBC (Schücking, 1879; von Korber, 1926). Accordingly, in the early 20th century there were many attempts to define standard ranges for red cell values in neonates (Chuinard, Osgood and Ellis, 1941; DeMarsh, Alt and Windle, 1948). It soon became evident that the red cell indices changed rapidly over the first few days and weeks so that very specific ranges would be needed relating to the exact postnatal age (Gairdner, Marks and Roscoe, 1951). The initial reference ranges were very wide, and it was apparent that, as well as postnatal age, many other factors influenced these values (Lanskowsky, 1960). The focus shifted to defining these factors, and on determining their effects.

Although the older reports were helpful in identifying some of the factors affecting the RBC, manual analysis was subject to considerable variation (Chuinard, Osgood and Ellis, 1941). Reference ranges derived from automated results were obtained on apparently healthy neonates with a capillary sample being taken on between 10 and 19 infants daily for a week and then less frequently for up to 12 weeks in a hospital where early cord clamping was standard (Matoth, Zaizov and Varsano, 1971). Reported parameters included haemoglobin, haematocrit, RBC and reticulocyte count. Other groups since then have also studied neonatal blood counts using automated analysers aiming to define standard reference values in different neonatal populations, with results usually being presented as the mean value and either one or two standard deviations. The relevant studies are summarised in Table 2-3. Reference ranges pertaining to cord blood have not been considered here, as cord blood sampling is not part of the study protocol.

The Intermountain Healthcare Hospitals have published three reports relating to RBC indices in neonates (Christensen et al, 2008; Jopling et al, 2009; Christensen et al, 2011). Neonates identified from their clinical records as having clinical features that suggested their RBC indices may not be considered normal were excluded, for example those diagnosed with anaemia, those who required a RBC transfusion, or associated with a history of placenta...
prævia or abruption. The most definitive report on the initial RBC values was published in 2008 and considered 17364 tests performed on 12016 neonates within 24 hours of birth (Christensen et al, 2008).

2.5.2 Factors affecting the red blood cell indices

By the 1940s it was evident that many factors influence neonatal red cell indices resulting in wide ranges (DeMarsh, Alt and Windle, 1948).

Timing of cord clamping

This was one of the first variables to be identified. Over a century ago Schücking calculated a lower blood volume in two infants whose cords were tied early, compared with 4 infants in whom the cord was not tied for several minutes (Schücking, 1879). Subsequently, DeMarsh reported that early cord clamping was associated with lower RCC and haemoglobin in babies tested in the first week of life compared with infants in whom clamping was delayed until the placenta had separated from the uterus (DeMarsh et al, 1941). This finding was confirmed in a study comparing values at 1 and 3 days in babies with immediate and delayed cord clamping (DeMarsh, Windle and Alt, 1942). Although, the accuracy of the values presented is doubtful as the calculation of total blood volume assumed that whole body and venous haematocrit are the same, the principle has been confirmed in modern studies using automated analysers. It is now well recognised that where clamping of the umbilical cord is delayed by >2 minutes from the time of birth a significant amount of extra blood is transferred from the placenta to the fetus resulting in an increase in total RCC.

Further, two systematic reviews and a meta-analysis of controlled trials also conclude that delaying umbilical cord clamping for a minimum of two minutes following birth has a benefit both immediately and into infancy (Hutton and Hassan, 2007; McDonald and Middleton, 2008). Specifically, delayed cord clamping was associated with a higher haematocrit, better iron status and a clinically significant reduction in the risk of anaemia. This association has also been demonstrated in preterm infants (Ultee et al, 2008). However, at present early cord clamping is standard in UK hospitals.
### Table 2-3. Summary of key studies reporting neonatal reference ranges for red blood cell indices.

<table>
<thead>
<tr>
<th>References</th>
<th>Number of neonates</th>
<th>Gestational age</th>
<th>Timing of sampling</th>
<th>Site of sample</th>
<th>Country</th>
<th>Indices reported</th>
<th>Manner reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1971 Matoth</td>
<td>32</td>
<td>term</td>
<td>weekly counts from 0-12 weeks</td>
<td>capillary</td>
<td>Israel</td>
<td>HB, HCT, RCC, MCV, MCHC, reticulocyte count</td>
<td>mean±1S, graphs against time (HB, RCC only)</td>
</tr>
<tr>
<td>1976 Zaizov</td>
<td>135</td>
<td>24 weeks -term</td>
<td>d1</td>
<td>capillary</td>
<td>Israel</td>
<td>HB, HCT, RCC, RDW, reticulocyte count</td>
<td>mean±1SD</td>
</tr>
<tr>
<td>1986 Ogala</td>
<td>99</td>
<td>term</td>
<td>d1; 1,2 and 6 weeks; 3, 6 months</td>
<td>venous</td>
<td>Nigeria</td>
<td>HB, HCT, RCC, MCV</td>
<td>mean±1SD, range</td>
</tr>
<tr>
<td>2000 Özbek</td>
<td>95</td>
<td>term</td>
<td>d1</td>
<td>capillary venous</td>
<td>Turkey</td>
<td>HB, HCT, RCC, MCV, MCH, MCHC, RDW</td>
<td>mean± 1SD,</td>
</tr>
<tr>
<td>2003 Kayiran</td>
<td>141</td>
<td>term</td>
<td>d7, d14, d21, d28</td>
<td>capillary venous</td>
<td>Turkey</td>
<td>HB, HCT, RCC, MCV, MCH, MCHC, RDW</td>
<td>mean± 1SD,</td>
</tr>
<tr>
<td>2006 Özyürek</td>
<td>77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>term</td>
<td>d7</td>
<td>venous</td>
<td>Turkey</td>
<td>HB, HCT, RCC, MCV, MCH, MCHC, RDW</td>
<td>mean±SE (range)</td>
</tr>
<tr>
<td>2008 Christensen</td>
<td>12016</td>
<td>22-42 weeks</td>
<td>d1</td>
<td>not recorded</td>
<td>North America</td>
<td>MCV, MCH, MCHC</td>
<td>mean± 1SD, graph with 95% CI, expected values by gestation (MCV, MCH)</td>
</tr>
<tr>
<td>2009 Jopling</td>
<td>39 559 (HB) 41957</td>
<td>22-42 weeks</td>
<td>at least once between d1-28</td>
<td>not recorded</td>
<td>North America</td>
<td>HB, HCT</td>
<td>mean± 1SD, graph with 95% CI by gestational age and postnatal age</td>
</tr>
<tr>
<td>2009 Christensen</td>
<td>39 559 (HB) 41957</td>
<td>22-42 weeks</td>
<td>at least once between d1-28</td>
<td>not recorded</td>
<td>North America</td>
<td>MHB, HCT, MCV, MCH, MCHC</td>
<td>graph with mean, 5&lt;sup&gt;th&lt;/sup&gt; and 95&lt;sup&gt;th&lt;/sup&gt; percentile by gestational age, smoothed curves for MCV, MCH</td>
</tr>
</tbody>
</table>

<sup>a</sup> This group included appropriate for gestational age and small for gestational age neonates; samples were also taken from 109 neonates on d1, but as these were cord blood samples they are not included here; <sup>b</sup> This paper summarises the work presented in Christensen 2008 and Jopling 2009, as well as papers on WBC and platelets. The only new information is smoothed curves giving reference ranges for MCV and MCH. Note that this table does not include papers which include babies who have received red blood cell transfusions (Obladen, Diepold and Maier, 2000); or where manual processing was used (Sasanakul et al, 1993).
Variations in neonatal RBC indices according to the source of the sample were first identified almost a century ago (Haden and Neff, 1924) when the haemoglobin and RCC of capillary and venous samples were compared. A series of investigations was reported by DeMarsh into the effect of the timing of cord clamping an apparent difference between capillary and venous samples was noted (DeMarsh et al, 1941; DeMarsh, Windle and Alt, 1942), leading to a study with the primary purpose of investigating the effect of early and late cord clamping, but with a secondary purpose of obtaining sagittal sinus samples and comparing these with the capillary sample results obtained in the initial study (DeMarsh, Alt and Windle, 1948). This showed that the haemoglobin and RCC values obtained from the sagittal sinus were lower than those reported from capillary samples. A third study, reported in the same paper, performed to compare capillary and sagittal sinus samples taken simultaneously from the same child, confirmed a higher RCC and haemoglobin in the capillary samples (DeMarsh, Alt and Windle, 1948).

Around the same time another group replicated these findings, also describing a higher haematocrit in capillary compared with venous samples from neonates (Vahlquist, 1941). This group also showed that the capillary haemoglobin fell after the first week, so that it was similar to the venous haemoglobin by the end of the second week. Subsequently, a study of capillary-venous differences beyond the first postnatal week, in 44 neonates of mixed gestational age and weight showed that although the values begin to approach one another with increasing postnatal age, mean capillary values still remain higher at 8-10 postnatal weeks (Rivera and Rudolph, 1982). This phenomenon was most marked in neonates with low birth weight (LBW) with the mean haemoglobin and mean haematocrit being 11-12% higher in capillary samples from those weighing <1500 grams at birth. However, by 8-10 weeks of age the mean haemoglobin and mean haematocrit were only 4-7% higher in the LBW group and only 3-5% higher in those with birth weight >2500 grams.

Similar findings were reported recently in paired venous and capillary samples from 95 healthy term newborns taken within 24 hours of delivery: haemoglobin, haematocrit and RCC were all significantly higher in capillary samples (Ozbek, Gürakan and Kayiran, 2000). The same group developed their work by taking simultaneous venous and capillary samples from a further 141 infants at all or some of the following time-points: 7, 14, 21 or 28 days old (Kayiran et al, 2003). Again, the venous-capillary differences were shown to narrow but still to persist over time, with higher haemoglobin, haematocrit and RCC in the capillary samples. Comparison of arterial and capillary or venous samples taken in a rapid sequential manner in
preterm babies indicates that arterial haemoglobin values are lower than either venous or capillary values, with the difference being the most pronounced between capillary and arterial values (Thurlbeck and McIntosh, 1987).

The mechanism underlying these differences is unclear although it may be partly accounted for by the movement of plasma from the capillary bed into the interstitial compartment resulting in local haemo-concentration consistent with the higher haemoglobin, haematocrit and RCC of capillary samples. Interestingly, the venous/capillary difference appears to become more marked in infants with neonatal sepsis which may well reflect compromise of the microcirculation with increased capillary leakage (Olvera-Hidalgo and Silva-Carrillo, 1976). Ideally therefore there would be separate reference ranges for venous, arterial and capillary samples. However, in practice this has been difficult to achieve as the largest data sets have been analysed retrospectively, and source of sample is poorly recorded on forms in clinical practice (Christensen et al, 2008; Jopling et al, 2009).

Postnatal age

A fall in haemoglobin with increasing postnatal age had been observed in reports from the first half of the last century (Vahlquist, 1941). The effect of postnatal age was specifically studied in 25 infants, with 105 venous samples, and also 102 bone marrow samples, being taken from birth to 3 months (Gairdner, Marks and Roscoe, 1951). Venous sampling was chosen as this was believed to be less subject to variation. The haemoglobin was seen to rise abruptly over the first day of life from ~18g/dl to ~20g/dl before falling to ~11-12g/dl in the eighth week and then remaining stable. A fall in the RCC and MCH was also observed after birth, but this was accompanied by a fall in MCV and so appeared less dramatic. The MCHC remained fairly constant throughout the 3 months. Comparison of these values with the erythroid activity of the bone marrow caused the authors to conclude that ‘erythropoiesis ceases when the oxygen saturation just after birth increases from about 65% in the umbilical vein to 95% just after birth’.

A similar pattern of postnatal changes in red cell values was confirmed and refined using automated counters in 1971 by Matoth and co-workers (Matoth, Zaizov and Varsano, 1971). Capillary samples were used throughout. This work suggested that the RCC nadir occurs during the 7th week, earlier than that of the haemoglobin, which was explained by the continuing fall in MCV. Importantly, Matoth also showed that erythropoiesis does not cease, but rather slows down in the immediate postnatal period. The reticulocyte count then increases from around the 5th week, but given the rapidly increasing weight and, blood volume, is initially unable to compensate adequately. The haemoglobin and RCC then rise
slowly from the 9th week, reflecting an increased red blood cell production, and the reticulocyte percentage falls to a steady-state. The authors note that the macrocytes, present at birth, are less dense than adult RBC, and suggest that these may be younger cells released early into the circulation during a period of increased antenatal erythropoiesis.

Following this, Stockman studied the natural history of red blood cell values in 40 preterm LBW infants (Stockman and Oski, 1980). The study states that the birth weights, all <1500 grams, were appropriate for gestation, so that prematurity rather than birth weight is the important variable. Capillary samples were taken at 1, 3 and 7 days of age and then weekly thereafter for the first seven weeks of life. A similar pattern to that seen in term infants was observed: the haemoglobin, RCC, MCV and MCH all fell with increasing postnatal age, suggesting that this, and not post-conceptual age, was the determining factor.

Finally, the most definitive work to date on red cell indices in the neonatal period reviewed the haemoglobin from 39559 and haematocrit from 41957 samples taken during the neonatal period (Jopling et al, 2009). Neonates born 29-34 weeks gestation and those born 35-42 weeks gestation were considered separately. In both groups the haemoglobin and haematocrit fell throughout the neonatal period. Importantly, in this very large dataset the rise in haemoglobin and haematocrit previously described in the immediate postnatal period (Gairdner, Marks and Roscoe, 1951) was not observed. Instead, a fall occurred during this period in the 29-34 week gestational age group, while values in the older group appeared to stay level before steadily falling from day 2. Early cord clamping to allow the preterm infant to be passed to a paediatrician, early use of intravenous fluid and consequent haemodilution, and frequent blood sampling may all play a part in the early fall in the younger group.

Birth weight, low birth weight or very low birth weight babies

Birth weight and gestation are closely related variables, but one cannot simply use either as a surrogate for the other. Low birth weight (LBW) is defined by WHO as weight < 2500 grams; while very low birth weight (VLBW) is defined as weight < 1500 grams. Only a few studies have compared results for babies of similar gestation but who were either appropriate for gestational age (AGA), or SGA.

A British study compared samples taken from birth to 14 days from 101 VLBW, AGA neonates with samples from 42 VLBW, SGA neonates (McIntosh, Kempson and Tyler, 1988). Initially, the SGA neonates had a higher haemoglobin and lower MCHC, whilst other red cell indices were similar. Results beyond the first day were difficult to interpret because of red blood cell transfusion. A more recent study compared cord blood samples from day 1 and
venous samples from day 7 from 73 term SGA neonates with 59 AGA term neonates (Ozyürek et al, 2006). Not all cases had samples at both time points. The SGA group initially had a higher haematocrit, RCC and MCV; but there were no significant differences in these by day 7. The MCHC remained lower in the SGA group at both time points.

Gestational age

The improved survival of babies born prematurely and/or at LBW, or even at very low birth weight (VLBW), has made the development of reference ranges for this population a pressing clinical need. The first study to investigate the effect of gestational age on red cell values in a systematic manner considered capillary samples from 125 neonates born from 24 weeks gestation to term (Zaizov and Matoth, 1976). Although the numbers for each gestational period are small there was no appreciable change in the haemoglobin or haematocrit over this period. However, the MCV gradually decreased from a mean 135 ± 0.2fl (mean ± SD) at 24-25 weeks gestation to 119 ± 9.4fl at term with a corresponding increase in the RCC from 4.65 ± 0.43 x10^{12}/l at 24-25 weeks to 5.14 ± 0.7 x10^{12}/l at term. There was a parallel decrease in the reticulocyte count with increasing gestational age from 6.0 ± 0.5% at 24-25 weeks to 3.2 ± 1.4% at term.

A retrospective review designed to define haematological values in healthy, VLBW infants and to study the effect of gestation and race was published in 2000 (Alur et al, 2000). Results of arterial full blood counts obtained within 3 hours of birth from 188 healthy neonates born ≤31 weeks gestation were reported. Babies deemed small for gestational age (SGA) were excluded. The analysis considered three gestational groups: 23-25 weeks; 26-28 weeks; and 29-31 weeks. Haemoglobin and haematocrit increased, while MCV and MCH decreased, with increasing gestational age. The RDW was similar for all three gestational groups.

One of the first in the series of large scale retrospective reports from the Intermountain Healthcare Hospitals reported results from 12 016 neonates ranging from 22 to 42 weeks gestation, and provides an analysis of MCV, MCH and MCHC by gestational age (Christensen et al, 2008). The MCV and the MCH decreased with advancing gestational age: the MCV falling from 119 ± 7fl (mean ± SD) at ≤ 25 weeks to 106 ± 4fl at 40 weeks. Thus, even at term, the MCV is much higher than in adults with the average neonatal MCV being higher than the upper limit of normal for adults (Bain, 2002) – as had been noted first by Wintrobe almost 90 years earlier (Wintrobe, 1929b). The MCH fell from 40 ± 2pg at 25 weeks gestation to 36 ± 2pg at 40 weeks. The MCHC did not vary significantly with gestation. In a review of their work the same group present these data afresh, with new graphs giving
mathematically smoothed curves for the MCV and MCH values by gestational age (Christensen et al, 2009). Finally, in the study of neonatal haemoglobin and haematocrit, reported by Jopling, the large dataset was broken down into those born 29-34 weeks gestation and those born at 35-42 weeks gestation (Jopling et al, 2009). Comparing values between the two groups the preterm infants initially had a lower haemoglobin and haematocrit. The fall in both was also more rapid in this group so that values throughout the 28 days remained lower than in the group of neonates born at 35-42 weeks gestation.

Additionally, there are several reports of haematological values in fetuses, sampled in utero in order to establish antenatal diagnosis, for example of toxoplasmosis or haemophilia. In the first, 409 pregnancies were studied (Forestier et al, 1986). Results from 163 term babies with preceding fetal samples were used to construct fetal reference values (mean ± SD) for four gestational groups: 18-20 weeks; 21-22 weeks; 23-25 weeks; and 26-30 weeks. Consistent with the more recent studies above, the HB and HCT rose with increased gestational age, while the MCV and MCH fell – the MCHC remained constant as the RCC increased. The same group have since reported haematological values from a much larger dataset of 2860 apparently normal fetuses (Forestier et al, 1991). Again the haemoglobin, haematocrit and RCC rose with increasing gestation, while the MCV fell.

Ethnicity

The impact of ethnicity is not clear. Red cell indices were reported for a Jamaican birth cohort of 243 children, none of whom had a haemoglobinopathy. Sequential samples taken from birth onwards showed similar values for haemoglobin, RCC, MCV and MCH as well as a similar postnatal pattern of change (Serjeant et al, 1980). However, differences were subsequently reported in two studies from Nigeria. In the first, cord blood samples were collected from 100 term neonates and processed manually (Abdurrahman and Adekoje, 1983). The haemoglobin and haematocrit were both lower than published comparable values in Caucasian neonates. The subsequent study, also from Nigeria, reported on venous samples from 99 healthy term babies taken at 1 day; 1, 2 and 6 weeks; 3 and 6 months (Ogala, 1986). At birth the mean values ± SD were haemoglobin 16.2 ± 1.7 g/dl; haematocrit 0.49 ± 0.05; RBC 4.88 ± 0.5 x10¹²/l; and MCV 101 ± 5.8 fl. A similar pattern of postnatal change to that already described for RBC values was reported. The lower absolute values of haemoglobin, haematocrit and RCC cannot be accounted for by sickle cell disease, as this was excluded. However, the frequency of carrier status for α or β thalassaemia is thought to be as high as 25% in this population (Kotila et al, 2009). The authors suggest that the lower
red cell values may be accounted for by lower socioeconomic status, endemic malaria, or racial differences.

Several studies have been able to compare neonates from different ethnic groups within the same general population. A retrospective review comparing arterial full blood counts obtained within 3 hours of birth in neonates born ≤31 weeks gestation in North America, reported statistically higher haemoglobin, haematocrit and MCV values in white compared with black infants (Alur et al, 2000). However, there is little information about potential socio-economic differences between the two groups. Subsequently, a study designed to establish normative data for black VLBW neonates reported similar ranges to those already established in white neonates – although some of the red cell indices, notably the haemoglobin, were at the lower limits of the established ranges (Stancheva et al, 2002).

Some of the best data on neonatal haematology reported from a non-Western country comes from a report by the HIV Prevention Trials Network who report haematological data from HIV uninfected women and their infants as a control group from five centres in Malawi, Tanzania and Zambia (Mwinga et al, 2009). They report haemoglobin and haematocrit taken at birth from 1900 healthy term neonates and processed on an automated analyser. The results are given as mean ± SD, and as a range with 95% CI: haemoglobin 18.5 ± 4.3g/dl (10.1-28.2) and haematocrit 0.548 ± 0.121 (0.297-0.802). In contrast to the previous reports these are higher than results typically reported for Western populations.

Nucleated red blood cells

It is not unusual to find nucleated red blood cells (NRBC) in the peripheral blood of neonates (Lippman, 1924), and they are variously reported per unit blood volume (/mm³ or /l) or per 100 WBC – the former being preferred due to variability in WBC. A recent review of NRBC in neonates concluded that a high values were associated with prematurity, increased erythropoiesis from chronic hypoxia, anaemia, maternal diabetes, bone marrow stress causing premature release for example in infection, and from postnatal hypoxia for example from acute blood loss (Hermansen, 2001). This also showed that the NRBC falls rapidly with increasing postnatal age, halving in the first twelve hours so that they have all but disappeared by the 3rd or 4th postnatal day. Subsequently, a prospective study set up to establish neonatal reference ranges for NRBC reported cord blood values from 455 premature and 240 term neonates. In addition, this confirmed that the NRBC falls with increasing gestation and birth weight (Perrone et al, 2005). The most recent reference ranges, and the largest data set, was published in 2010 after a retrospective analysis of 35 396 neonatal computer records (Christensen et al, 2011).
2.6 White blood cell parameters in neonates

‘Blood leucocyte counts in newborn babies have generally been considered to be so variable and unpredictable as to be of little value for clinical purposes’

Marietta Xanthou, Neonatal Research Unit, Institute of Child Health, London. 1972

‘special efforts [should] be made to develop leukocyte reference values for all ages, but especially in the pediatric age groups including neonatal and premature infants’

Hematology Resource Committee of the College of American Pathologists. 1978

2.6.1 Development of reference ranges

Of all the haematological parameters studied in neonates, the white blood cell (WBC) count and its differential have probably generated the most interest clinically – to the extent that the reference ranges are often referred to by name. However, initial attempts to generate reference data were problematic. One of the first of these was a study of the WBC and differential for 1081 children aged from birth to 15 years (Kato, 1935). This showed a very high WBC and neutrophil count at birth which increased and peaked within 24 hours, before falling rapidly in the first few days. The pattern for the rest of the neonatal period was quite variable, reflecting low numbers of neonates sampled at each stage. Another study, published in the same year also showed a wide variation in the WBC during the neonatal period, with a reported range of 5-24 x10^9/l (Washburn, 1935). The reported rapid changes with age, and the very wide ranges, caused some highly regarded authors in the field of neonatal haematology to conclude that attempts to define reference ranges in neonates were ‘not only unnecessary here but might be more confusing than valuable’ (Smith, 1959; Wintrobe, 1967).

An account of the total and differential WBC by Xanthou was published in 1970 (Xanthou, 1970). This reported serial counts, analysed on an automated counter, on 55 healthy term neonates, and a further 14 neonates with a birth weight <2500 grams – 13 of whom were born at <39 weeks gestation. This was followed by a subsequent report of the WBC in ill neonates (Xanthou, 1972).

The seminal paper defining reference ranges for normal blood neutrophil concentrations was reported in 1979 using data from 304 healthy neonates and 130 with perinatal complications unlikely to affect neutrophil count (Manroe et al, 1979). These are still referred to by name as the ‘Manroe charts’. In addition to providing reference data, this paper demonstrated the importance of the differential WBC – particularly neutropenia – in identifying neonates at risk
of bacterial disease and convincingly stated the case for the role of WBC analysis in the management of sick neonates. Later the same group published neonatal reference ranges for lymphocyte, monocyte and eosinophil counts from the same cohort (Weinberg et al, 1985), and then for neutrophil counts in a population of VLBW neonates (Mouzinho et al, 1994). These have both been widely regarded as the benchmark reference ranges for the respective populations. However, this place has recently been challenged by a publication from the Intermountain Healthcare Hospitals which set out to compare the Manroe and Mouzinho ranges in a much larger sample size and using modern cell counting instruments (Schmutz et al, 2008). In addition, they aimed to investigate the effect of high altitude. The study reported results from 30 354 tests performed on neonates of 23-42 weeks gestation between 1 January 2004 and 31 May 2007. They excluded neonates who were in a group known to be associated with an increased frequency of abnormal neutrophil counts, namely: those born to mothers with pregnancy induced hypertension; those with identified early onset bacterial sepsis; those with a subsequent diagnosis of congenital neutropenia; and those with trisomy 13, 18 or 21. The ranges published by Schmutz are given as the 5th percentile value, the mean value and the 95th percentile value in order to offer the clinician a readily understandable expected range. In contrast the Manroe and Mouzinho papers show the entire range of values as scatter plots. Although neutrophilia would be diagnosed at a higher count, for example >13 x10^9/l at 72h compared with >7 x10^9/l using Manroe, neutropenia would be diagnosed similarly whether using Manroe or Schmutz for term and near term infants which agree on a diagnosis of neutropenia at a neutrophil count <2.5 x10^9/l after 72h or using Mouzinho or Schmutz for preterm neonates, at a neutrophil count <1 x10^9/l.
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<tr>
<th>References</th>
<th>Number of neonates</th>
<th>Gestational age</th>
<th>Timing of sampling</th>
<th>Site of sample</th>
<th>Country</th>
<th>Indices reported</th>
<th>Manner reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970 Xanthou</td>
<td>69</td>
<td>55 term</td>
<td>every 6h for 24h (72h if term), daily until at least d20 (d10 if term)</td>
<td>capillary</td>
<td>UK</td>
<td>neutrophil, metamyelocytes, eosinophils, basophils, lymphocytes, monocytes</td>
<td>mean±1SD , graphs of mean±1SD, range v postnatal age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 preterm</td>
<td></td>
<td></td>
<td></td>
<td>eosinophil</td>
<td>v time from nadir weight</td>
</tr>
<tr>
<td>1979 Gibson</td>
<td>38</td>
<td>27-35</td>
<td>at least once between d1-45</td>
<td>capillary</td>
<td>North America</td>
<td>eosinophil</td>
<td>mean±2SD graphs of range v postnatal age</td>
</tr>
<tr>
<td>1979 Manroe</td>
<td>304a</td>
<td>26-44</td>
<td>at least once between d1-28</td>
<td>most capillary, few venous</td>
<td>North America</td>
<td>WBCc, neutrophils, I:T</td>
<td>graphs of range v postnatal age</td>
</tr>
<tr>
<td>1981 Bhat</td>
<td>45</td>
<td>&lt;37</td>
<td>d1, then twice weekly until discharge</td>
<td>not specified</td>
<td>North America</td>
<td>eosinophil count</td>
<td>graphs of mean, SD and range by postnatal age</td>
</tr>
<tr>
<td>1981 Lloyd</td>
<td>24</td>
<td>&lt;33</td>
<td>1,12,24,48,72,96,120 hours</td>
<td>capillary</td>
<td>UK</td>
<td>mature and band neutrophils</td>
<td>mean, median, range</td>
</tr>
<tr>
<td>1984 Weinberg</td>
<td>210a</td>
<td>36 term</td>
<td>at least once between d1-28</td>
<td>capillary</td>
<td>North America</td>
<td>monocytes, lymphocytes, eosinophils</td>
<td>5,50and95 percentile values graphs of range v postnatal age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>174 preterm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1985 Scott-Emuakpor</td>
<td>182</td>
<td>107 term</td>
<td>d1, d7, 4th week</td>
<td>venous</td>
<td>Nigeria</td>
<td>WBCc, neutrophils, band forms, I:T, monocyte, lymphocyte, eosinophil, basophil</td>
<td>WBC given as mean± 1SD differential values are given as mean and range</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 preterm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>35 post-term</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1994 Mouzinho</td>
<td>135</td>
<td>29.9±2.3 (1SD)</td>
<td>at least once between d1-28</td>
<td>capillary</td>
<td>North America</td>
<td>mature and immature neutrophils, I:T</td>
<td>mean±1SD</td>
</tr>
</tbody>
</table>

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Table 2-4. Summary of relevant reports of white blood cell count and differential counts in the general neonatal population.
<table>
<thead>
<tr>
<th>References</th>
<th>Number of neonates</th>
<th>Gestational age</th>
<th>Timing of sampling</th>
<th>Site of sample</th>
<th>Country</th>
<th>Indices reported&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Manner reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995 Schelonka</td>
<td>193</td>
<td>term</td>
<td>d1</td>
<td>capillary</td>
<td>North America</td>
<td>Mature and immature neutrophils, I:T</td>
<td>mean, range with 10-90% CI</td>
</tr>
<tr>
<td>2000 Özbek</td>
<td>95</td>
<td>term</td>
<td>d1</td>
<td>capillary venous</td>
<td>Turkey</td>
<td>WBC&lt;sup&gt;b&lt;/sup&gt;, neutrophils, monocyte, lymphocyte, eosinophil, basophil, metamyelocyte, myelocyte</td>
<td>mean± 1SD,</td>
</tr>
<tr>
<td>2003 Kayiran</td>
<td>141</td>
<td>term</td>
<td>d7, d14, d21, d28</td>
<td>capillary venous</td>
<td>Turkey</td>
<td>WBC&lt;sup&gt;b&lt;/sup&gt;, neutrophils, I:T, monocyte, lymphocyte, eosinophil, basophil, metamyelocyte, myelocyte</td>
<td>Whether different in capillary cf venous sample only, no numerical value given mean±SE (range)</td>
</tr>
<tr>
<td>2006 Özyürek</td>
<td>77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>term</td>
<td>d7</td>
<td>venous</td>
<td>Turkey</td>
<td>WBC, neutrophils, I:M, I:T, monocytes, eosinophils, basophils, metamyelocytes, lymphocytes,</td>
<td></td>
</tr>
<tr>
<td>2008 Schmutz</td>
<td>30354</td>
<td>22-42 wks</td>
<td>at least once between d1-10</td>
<td>not recorded</td>
<td>North America</td>
<td>Neutrophil, immature neutrophil</td>
<td>mean± 1SD, graph with 95% CI, expected values by postnatal age</td>
</tr>
<tr>
<td>2009 Mwinga</td>
<td>1897</td>
<td>term</td>
<td>birth, 4-6 weeks</td>
<td>not recorded</td>
<td>Malawi, Tanzania, Zambia</td>
<td>WBC</td>
<td>mean± 1SD, and range with 95% CI,</td>
</tr>
<tr>
<td>2009 Christensen&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;30 354</td>
<td>22-42 wks</td>
<td>at least once between d1-10</td>
<td>not recorded</td>
<td>North America</td>
<td>Neutrophil</td>
<td>graph with mean, 5&lt;sup&gt;th&lt;/sup&gt; and 95&lt;sup&gt;th&lt;/sup&gt; percentile by gestational age, smoothed curves for MCV, MCH</td>
</tr>
</tbody>
</table>

a. This study also reported on a further group of babies with perinatal complications.
b. This group included appropriate for gestational age and small for gestational age neonates; samples were also taken from 109 neonates on d1, but as these were cord blood samples they are not included here.
c. Italics indicate that this parameter was obtained by manual methods. Regular type indicates that this parameter was obtained using an automated analyser.
I:M immature : mature neutrophil ratio; I:T immature : total neutrophil ratio
Postnatal age

Postnatal age was identified early on as a key factor affecting the WBC – and it was partly the rapid changes reported in the first few days of life that caused some to be suspicious of the clinical utility of the WBC. In a cross-sectional study of WBC in children Kato found a high neutrophil count at birth, estimated mean at ~ 12.5 x 10^9/l (Kato, 1935). Moreover, the neutrophil count, and hence the WBC, then increased further peaking between 12 and 24 hours with estimated mean neutrophil count ~15 x 10^9/l. This then fell rapidly to a nadir at around the 4th postnatal day and the pattern for the rest of the neonatal period was quite variable, which probably reflects the small numbers of neonates sampled at each stage.

In the paper by Xanthou the neutrophil count rose in all cases reaching a peak at 12 hours, followed by a gradual fall and then remaining stable from around the 10th day (Xanthou, 1970). The mean neutrophil count at birth and 12 hours in term compared with LBW babies respectively were 8 x 10^9/l and 13 x 10^9/l, compared with 5 x 10^9/l and 8 x 10^9/l. The dramatic early increase in neutrophils is not accompanied by an increase in neutrophil precursors and is presumed to represent demargination of existing neutrophils.

Manroe similarly reported a peak neutrophil count at 12-14 hours, then a fall to the 5th postnatal day and a plateau thereafter (Manroe et al, 1979). The values are given graphically as a range with 95% CI. The range of the neutrophil count during this plateau phase was very similar to that given by Xanthou: 1.8-5.4 x 10^9/l compared with 1.8-5.5 x 10^9/l. Schmutz found that the timing of the peak neutrophil count varied with gestational age: occurring at 6-8h in those born ≥ 28 weeks gestation compared with 24h if born <28 weeks gestation. In both groups the neutrophil count gradually fell over the first 72 hours of life, remaining stable thereafter (Schmutz et al, 2008).

Sample site

A comparison of the WBC from arterial, venous and capillary samples taken from the same healthy term neonates in a rapid sequential manner found that the mean arterial WBC and venous WBC were respectively 77% and 82% of the mean capillary value (Christensen and Rothstein, 1979). This relationship was confirmed in two subsequent studies. In the first, 60 paired venous and capillary samples were taken from 30 healthy term neonates on the first and second day of life (Peevy, Grant and Hoff, 1982). On both days the neutrophil count was significantly higher in capillary samples. Similarly, in a study of 13 paired venous-capillary and 21 paired venous-arterial samples, the neutrophil count was respectively higher in
capillary than venous, and higher in venous than arterial samples (Thurlbeck and McIntosh, 1987). A more recent study of capillary-venous differences in the full blood counts of 95 healthy term newborns taken within 24 hours of delivery reported a higher WBC in capillary compared with venous samples (Ozbek, Gürakan and Kayiran, 2000). When the WBC differential was analysed it became apparent that the increase in capillary WBC was accounted for by an increase in neutrophils, which may tend to demarginate in the microcirculation.

The main flaw in the three main papers on WBC reference ranges is that the source of the sample was not recorded (Manroe et al, 1979; Mouzinho et al, 1994; Schmutz et al, 2008). However, Schmutz considered that very few counts were likely to have been derived from arterial samples so that given the very large number of samples the effect was likely to be small.

High altitude

Studies examining the effect of high altitude have consistently reported that neutrophil counts increase with altitude (Carballo et al, 1991; Maynard, Reed and Kircher, 1993; Schmutz et al, 2008). The Intermountain Hospitals group suggest that this explains the higher upper limit they report as this is similar to the two earlier studies of neonates at high altitude (Carballo et al, 1991; Maynard, Reed and Kircher, 1993), despite differences in counting methods, but higher than the upper limit of the Manroe study which was at low altitude (Manroe et al, 1979). The mechanism underlying this is not understood, although it presumably relates to reduced oxygen delivery to the fetus at high altitude.

Sex

In contrast to previous studies, which had typically reported that there was no variation with sex, Schmutz found that neutrophil counts were significantly higher in females, although the reasons for this are unclear (Schmutz et al, 2008).

Ethnicity

It was recognised in the 1970s that neutrophil counts were lower in black compared with white Americans (Karayalcin, Rosner and Sawitsky, 1972) – but, it is not clear whether this is also true for neonates. A Nigerian study reported serial WBC taken during the neonatal period from 107 term, 40 preterm and 35 post-term neonates (Scott-Emuakpor et al, 1985). Manual differential counts were done on 85, selected at random. The mean WBC was high at 12.6 x10⁹/l, with no difference between the groups of different gestational ages. However, differential analysis indicated that the mean neutrophil count was lower at 5.7 x10⁹/l and the
mean lymphocyte count higher at 5.1 $\times 10^9$/l than comparable values reported in Western populations. In contrast, a study which compared the differential WBC of cord blood from 242 neonates from five different ethnic groups reported no difference between any of the ethnic groups, and concluded that the differences observed in adults are acquired later (Chan, Hayes and Bain, 1985). This is consistent with the very large Intermountain Healthcare Hospitals analysis which found no variation in the differential WBC count either between black and non-black, or Hispanic and non-Hispanic (Schmutz et al, 2008).

Stress

In a study designed to identify factors that might affect the WBC of neonates, Christensen, who almost 30 years later would be one of the pioneers of the Intermountain Healthcare Hospitals work on neonatal reference ranges, considered source of sample and the effect of crying (Christensen and Rothstein, 1979). Arterial, venous and capillary samples were taken in a rapid sequential manner from neonates who had been ‘resting quietly for an hour’. Sampling was then repeated after one of three procedures likely to induce crying: circumcision, chest physiotherapy or heel prick testing. The WBC increased after crying. The increase was most pronounced in neonates who had demonstrated violent crying, for example following circumcision, with a specific increase in immature neutrophils.

Labour is considered to be stressful for the neonate, as well as for the mother. Schmutz reported significantly higher neutrophil counts at 18 hours in neonates born after labour compared with those delivered by a labour-free caesarean section (Schmutz et al, 2008). The ratio of immature to total neutrophil count did not increase suggesting neutrophil demargination occurring as a rapid response to the release of stress induced catecholamines.

Maternal pregnancy-induced hypertension

Manroe found that 57% of neonates whose mothers had had hypertension during pregnancy had neutrophil counts below the reference range of the control neonates, while no values exceeded the reference range (Manroe et al, 1979). This effect appeared to be limited to the first 72 hours, and was independent of gestation or birth weight. The association between maternal hypertension and neonatal neutropenia has been confirmed in subsequent reports (Koenig and Christensen, 1989; Juul, Haynes and McPherson, 2004).

Early-onset bacterial sepsis

Two years after her work on WBC reference ranges in term and preterm neonates, Xanthou published the serial WBC counts of 35 ill preterm and term neonates taken throughout the
neonatal period (Xanthou, 1972). The total WBC was not informative, but importantly, they found that in those with proven or suspected bacterial infection the absolute neutrophil count increased. However, on closer inspection this was due to an increase of immature neutrophils or band forms and masked a significant fall in mature neutrophils to < 1.0 x10⁹/l. The eosinophil count also fell in those with proven or suspected bacterial infection to 0 x10⁹/l. Published immediately back-to-back with this report was a paper from Newcastle similarly describing neutropenia in three neonates with fatal bacterial sepsis (Gregory and Hey, 1972).

The study by Manroe identified neutropenia (a neutrophil count below the reference range determined for healthy neonates in the same paper) as being the most important predictor of infection, with 77% neutropenic neonates having suspected or confirmed bacterial infection (Manroe et al, 1979). In contrast, neutrophilia (a neutrophil count above the reference range determined for healthy neonates in the same paper) was less specific with only 58% neonates having suspected of confirmed bacterial infection. Further, it was shown that an increase in the immature: total neutrophil ratio (I:T), and not the absolute number of immature forms, was predictive of infection. The utility of the Manroe ranges in identifying neonates with sepsis was validated in subsequent reports (Benuck and David, 1983; Rodwell, Leslie and Tudehope, 1988). However, it must be noted that Rosenfeld, the Professor of Pediatrics in the unit at the University of Texas which generated the Manroe ranges, was quick to emphasise that they had ‘never reported or suggested a “sepsis-scoring system”’ (Rosenfeld, 1995). He believed that the ranges were useful to assess serial FBCs in conjunction with the clinical course to inform decisions about discontinuing antibiotics, rather than in helping to diagnose neonatal sepsis.

The recognition that neutropenia was frequent in healthy preterm babies when their counts were considered against the Manroe ranges, led to the publication of specific ranges for preterm neonates. A study of the comparative usefulness of the Manroe and Mouzinho ranges in the diagnosis of sepsis in VLBW neonates was published – again by the same group – in 1997 (Engle et al, 1997). This found that although deviations from the original Manroe ranges were more sensitive predictors of neonatal sepsis, deviations from the Mouzinho ranges were more specific. Additionally, this paper suggested that the association with neutropenia and infection was most important in the first week of life, after which neutrophilia was more common with an increase in immature neutrophils and I:T. Where neutropenia was associated with infection later on in the neonatal period it tended to be associated with Gram-negative infection.
Gestational age

The study of WBC by Xanthou, had considered healthy term and premature babies, but differences between the groups were not described in detail and only 14 premature babies were included (158). With the increasing survival of neonates at early gestation, it became apparent that, even in the absence of infection, preterm neonates often appeared to be neutropenic when considered against the Manroe charts (Lloyd and Oto, 1982; Mouzinho et al, 1992). This led to the determination of reference ranges for neutrophil counts in 63 preterm VLBW neonates with a mean gestational age of 29.9 weeks (Mouzinho et al, 1994). These confirmed that the neutrophil count was lower, and the ranges wider. Interestingly, the reference ranges for immature neutrophils and I:T were very similar to the earlier reports from older preterm and term neonates.

2.6.3 Specific considerations: eosinophils, monocytes, basophils and lymphocytes

Eosinophils

An increase in the eosinophil count at one week and also an increase in the eosinophil count of premature compared with term neonates were described in the 1950s (Medoff and Barbero, 1950; Burrell, 1952). In the paper by Xanthou the eosinophil count varied between healthy term and LBW neonates (Xanthou, 1970). In term neonates the mean fell from >0.8 x10^9/l in the first few days to 0.5 x10^9/l from the 4th day until the end of the neonatal period. The range also narrowed during this time. In the LBW group the mean eosinophil count was <0.6 x10^9/l during the first week, rising to just over 1.0 x10^9/l at the end of the neonatal period.

In a subsequent study of ill neonates, Xanthou reported eosinopenia in neonates with proven or probable infection (Xanthou, 1972).

A study of the natural history of the eosinophil count in 38 healthy neonates all born <36 weeks gestation found an eosinophilia (defined as eosinophil count >0.7 x10^9/l) in over three quarters of those studied (Gibson, Vaucher and Corrigan, 1979). The eosinophil count tended to rise from ~day 19, reaching a peak after a week. However, in those who had an infection this increase was halted and the eosinophil count was low. A prospective study of 45 premature neonates reported an association with eosinophilia and smaller, preterm neonates, parenteral nutrition, endotracheal intubation and blood transfusions (Bhat and Scanlon, 1981).

Definitive reference ranges were published by Weinberg, reporting data from 848 counts taken from 479 neonates (Weinberg et al, 1985). The reference ranges were given as the 5th-95th percentile values for 0-60 hours, 61-120 hours and 121-720 hours. These were
compared with counts from three groups of neonates who had a clinical condition known to affect the neutrophil count: 82 neonates with ABO incompatibility; 68 neonates with maternal hypertension; and, 140 with suspected or proven sepsis. There were no significant differences reported with sex, birth weight or gestational age. Eosinophil values were constant throughout the neonatal period, and consistent with earlier reports there was an early fall in eosinophils in those with suspected or proven sepsis.

Finally, in their largest analysis to date the Intermountain Healthcare Hospitals group have published reference ranges based on 63 371 counts from neonates from 22 to 42 weeks gestation, taken throughout the neonatal period (Christensen et al, 2010). The results were given as mean and 5-95% range. These values for the counts on day 1 increased linearly with increasing age, with mean (range) for a 40 week gestation neonate of 0.55 x10^9/l (0.14 – 1.3). The values were stable throughout the neonatal period, with only a slight increase in the upper limit for the 4th week.

Monocytes

The monocyte count appears to be comparatively stable throughout the neonatal period, although several reports have suggested that it is slightly higher in the first 24 hours and then falls slightly over the first week, remaining stable thereafter (Xanthou, 1970; Weinberg et al, 1985). Monocyte counts were higher in neonates with suspected or proven sepsis, typically occurring during the recovery phase at the end of the neonatal period (Weinberg et al, 1985). The same study reported an increased monocyte count in the first few days in neonates with ABO incompatibility.

Xanthou reported a slight rise in monocytes during the first 12 hours of life followed by a fall until the 3rd day and then a rise to the 7th day before levelling out from day 10 in both groups at ~ 1.0 x10^9/l, considerably higher than in adults (Xanthou, 1970). The reference range produced by Christensen describes a linear increase in monocyte count with increasing gestational age (Christensen et al, 2010). On the first day of life in a 40 week gestation neonate the mean (range) monocyte count was 1.4 x10^9/l (0.3-3.3). The monocyte count increased slightly during the first two weeks of life.

Lymphocytes

The reference data published by Weinberg (Weinberg et al, 1985) was consistent with earlier reports (Kato, 1935; Xanthou, 1970) with regard to both value and pattern with a fall from the 3rd day to the 5th day then rising to plateau at a slightly higher value than initially. The 5th percentile value was lower and the 95th percentile higher for neonates with suspected or
proven sepsis. There was also a lower lymphocyte count in children born to hypertensive mothers, but this effect only lasted for the first few days.

Basophils

There is little reported on the basophil count of neonates. Xanthou reported that the pattern of change of basophils over time resembled that of eosinophils and stated that the number of basophils was low, but did not provide any figures (Xanthou, 1970). There is an early, but very detailed report of the basophil count in 20 neonates on samples taken at birth, half an hour, 4 hours, 24 hours, then at 2, 3, 4, 5 and 7 days (Mitchell, 1955). This reported a rapid rise in the basophil count over the first 24 hours followed by a rapid fall. The peak count at 24 hours was $0.52 \times 10^9/l \pm 0.28$ (mean ± SD).
2.7 Platelet parameters in neonates

For many years attempts to define neonatal reference ranges for platelets were hampered by inadequate manual techniques with very wide reported references ranges which were consequently of little clinical value (von Farnos, 1926). In 1950 Brecher and Cronkite published a landmark paper, describing a demonstrably reproducible technique that is still used today as the standard means of obtaining a manual platelet count (Brecher and Cronkite, 1950). The use of this technique to define reference ranges for neonates was reported in 1961 using modified phase microscopy on 204 paired capillary samples taken within 96 hours of birth from 105 healthy term neonates (Ablin et al, 1961). The count was determined for each sample and the average for each pair was taken. Although the trend was towards an increase with postnatal age, this was not significant, and the reported mean ±2SD for the period was 200 ± 100 x10^3/mm^3. In 1973 Sell published reference data for a variety of haemostatic factors based on samples taken in the first 36 hours from 106 healthy neonates of varying gestational age, with a reported mean platelet count for term neonates of 300x10^3/mm^3.

Automated counters improved accuracy further, although early analysers which relied on differentiating RBC from platelets according to size only, typically using a cut-off of 40 fl, would erroneously count giant platelets as RBC. This would falsely increase the RCC – and thus the MCHC, whilst reducing the MCH – whilst giving a falsely low platelet count. This problem has been addressed by more modern analysers which additionally use laser light scattering, flow cytometry and optical impedance to distinguish between RBC and platelets. The Intermountain Healthcare Hospitals have published neonatal reference data for platelets, based on the records of over 47 000 patients (Wiedmeier et al, 2009). This report is taken as the definitive reference data set.

The most relevant reports of platelet values in apparently healthy neonates are summarised in Table 2-5.
Table 2-5. Summary of relevant reports of reference platelet values in the general neonatal population.

<table>
<thead>
<tr>
<th>References</th>
<th>Number of neonates</th>
<th>Gestational age</th>
<th>Timing of sampling</th>
<th>Site of sample</th>
<th>Country</th>
<th>Indices reported</th>
<th>Manner reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987 Patrick</td>
<td>143</td>
<td>24 weeks - term</td>
<td>d1</td>
<td>venous</td>
<td>North America</td>
<td>PLT count, MPV, PDW</td>
<td>mean±1SD</td>
</tr>
<tr>
<td>2000 Özbek</td>
<td>95</td>
<td>term</td>
<td>d1</td>
<td>capillary</td>
<td>Turkey</td>
<td>PLT count, MPV</td>
<td>mean± 1SD</td>
</tr>
<tr>
<td>2003 Kayiran</td>
<td>141</td>
<td>term</td>
<td>d7, d14, d21, d28</td>
<td>capillary</td>
<td>Turkey</td>
<td>PLT count, MPV</td>
<td>mean± 1SD</td>
</tr>
<tr>
<td>2006 Özyūrek</td>
<td>77ᵃ</td>
<td>term</td>
<td>d7</td>
<td>venous</td>
<td>Turkey</td>
<td>PLT count, MPV</td>
<td>mean±SE (range)</td>
</tr>
<tr>
<td>2009 Mwinga</td>
<td>1893</td>
<td>term</td>
<td>birth, 4-6 weeks</td>
<td>not recorded</td>
<td>Malawi, Tanzania, Zambia</td>
<td>PLT</td>
<td>mean± 1SD, and range with 95% CI,</td>
</tr>
<tr>
<td>2009 Wiedmeier</td>
<td>47291</td>
<td>22-42 weeks</td>
<td>at least once between d1-90</td>
<td>not recorded</td>
<td>North America</td>
<td>PLT count, MPV</td>
<td>mean± 1SD, graph with 95% CI by gestational age and postnatal age graph with mean, 5ᵗʰ and 95ᵗʰ percentile by gestational age, smoothed curves for MCV, MCH</td>
</tr>
<tr>
<td>2009 Christensenᵇ</td>
<td>Not stated</td>
<td>22-42 weeks</td>
<td>at least once between d1-28</td>
<td>not recorded</td>
<td>North America</td>
<td>PLT count, MPV</td>
<td></td>
</tr>
</tbody>
</table>
2.7.1 Factors affecting neonatal platelet indices

Postnatal age

Both studies using the Brecher and Cronkite method on healthy term (Ablin et al, 1961) and healthy premature neonates showed a trend for the platelet count to increase during the neonatal period (Aballi, Puapondh and Desposito, 1968). A similar rise in platelet count over the first 10 days of life was reported for venous samples from 60 healthy neonates with birth weight < 2500 grams (Appleyard and Brinton, 1971), and in a further group of 155 healthy neonates (Arad et al, 1986). Another study comparing venous and arterial platelet counts in AGA and SGA neonates with birth weights <1000 grams reported a nadir at around the 4-5th postnatal day, before rising with age (McIntosh, Kempson and Tyler, 1988). The nadir in the SGA neonates was just below 100 x10^9/l.

One of the few non-Western papers is a prospective study of venous platelet counts in healthy term neonates in Thailand (Sasanakul et al, 1993). This relied on the Brecher and Cronkite method, rather than automated counting. There is a high incidence of haemoglobinopathy in this population, but as a visual methodology was used, it is assumed that cases with haemoglobinopathies were excluded (Wanapirak et al, 2004; Nathalang et al, 2005). Samples were analysed by postnatal age, and showed an initial plateau at ~280 x10^9/l before a rise and then a slight fall. However, the time intervals range from 12 hours to 7 days so that the exact relationship with postnatal age is hard to determine. A much larger non-Western study set up to look at the impact of HIV, reported platelet counts of 240.8 ± 102.7 x10^9/l (mean ±SD) with range 76 – 458 x10^9/l (95% CI) in 1893 healthy control neonates based on samples taken at birth and using an automated counter (Mwinga et al, 2009).

A rise in platelet count during the neonatal period was demonstrated in a study comparing venous-capillary differences in 141 term neonates sampled at weekly intervals (Kayiran et al, 2003), and in another study by the same group comparing SGA with AGA term neonates (Ozyürek et al, 2006). However, the definitive contribution comes from the report by the Intermountain Healthcare Hospitals (Wiedmeier et al, 2009). This shows an increased platelet count with increasing postnatal age to a peak at around day 15, followed by a slight fall to day 28. The data is provided graphically: the mean count at birth is ~250 x10^9/l, rising to ~420 x10^9/l and then falling to ~320 x10^9/l at day 28.
Gestational age

In response to a mixture of reports comparing term with premature neonates indicating similar (Fogel, Arias and Kung, 1968), slightly lower (Wolff and Goodfelllow, 1955) and very much lower platelet counts (Medoff, 1964) for the latter, a larger study reported results of 298 healthy premature LBW and VLBW neonates, and 88 healthy term controls, using the Brecher and Cronkite method (Aballi, Puaponhd and Desposito, 1968). Samples were taken within the first 48 hours of life, and repeated during the second and fourth weeks for some of the premature neonates only. The platelet count in the premature neonates was significantly lower (P<0.05) than in term neonates over the first 48 hours; but with a mean of 220 x10^3/mm^3 was not as dramatically low as some earlier reports had suggested. Further, the authors concluded that a platelet counts of <100 x10^3/mm^3 was an abnormal finding in a premature neonate. The exact gestational ages are not given in the above studies.

However, in a study looking at a variety of haemostatic factors in 106 healthy neonates, the platelet count within the first 36 hours was analysed by gestational age: 27-30 weeks; 31-33 weeks; 34-36 weeks; 37-39 weeks and 40-42 weeks (Sell and Corrigan, 1973). Interestingly, the platelets counts were similar for all gestational groups, with no significant variation. The results are given graphically only, and appear to have a mean of between 250 and 300 x10^3/mm^3. For many years these were regarded as standard reference data. However, the numbers are small with only 8 neonates in most premature group.

The reports of haematological values in fetuses mentioned above also included platelet counts. In the first study of 163 fetuses, reference values are given for four gestational groups: 18-20 weeks; 21-22 weeks; 23-25 weeks; and 26-30 weeks. There were no significant differences in platelet counts between these groups, with mean values of 242-259 x10^9/l at all gestations (Forestier et al, 1986). The subsequent larger dataset of 2860 apparently normal fetuses reported similar mean values of 232-247 x10^9/l in fetuses from 18 weeks gestation onwards (Forestier et al, 1991). The largest dataset of over 47 000 neonates ranging from 22-42 weeks gestation, reported that the platelet count rose with increasing gestational age from mean ~ 200 x10^9/l at 22 weeks to ~250 x10^9/l at term (Wiedmeier et al, 2009).

There are a few studies which have considered platelet size and gestational age. A study of 27 term and 10 premature neonates did not show a significant difference in mean platelet volume between these groups, being 8.47 fl and 8.08 fl respectively (Kipper and Sieger, 1982). This study did show an association with thrombocytopenia and increasing mean platelet volume. However, a larger study of 78 term and 65 premature neonates did show a
significant difference with a mean platelet volume of 9.06 ±0.82 fl (mean ± SD) for term and 8.51 ±0.97 fl (mean ± SD) for premature infants. This conflicts with a study of 101 fetal blood samples taken in utero at intervals between 8-40 weeks gestation and using flow cytometry to calculate platelet size which demonstrated a fall in mean platelet volume with increasing gestational age (Meher-Homji et al, 1994).

Birth weight

A report of venous samples taken from 60 healthy neonates <2500 grams at days 1, 3, 5, 7, 10, 14, 21 and 28 days found similar results to term neonates (Appleyard and Brinton, 1971). Another study compared results, taken during the first 14 days, from 143 neonates with birth weight <1000 grams: 101 were AGA, 42 were SGA (McIntosh, Kempson and Tyler, 1988). The mean platelet count was significantly lower in the SGA neonates. The results are only given graphically, but indicate an initial count of >200 x10^9/l in the AGA compared with ~130 x10^9/l in the SGA group. In both groups the platelet count fell to a nadir at around the 4-5th postnatal day, before rising with age. The nadir in the SGA was just below 100 x10^9/l.

Another study designed to determine neonatal reference ranges for MPV, also reported on platelet count, and found a significantly higher count in 119 neonates with birth weight >2000 grams at 323 ± 107 x10^3 /mm^3 (mean ± SD) compared with 284 ± 114 x10^3 /mm^3 (mean ± SD) in 36 neonates with birth weight <2000 grams (Arad et al, 1986). A recent study compared venous counts in 64 SGA neonates with 45 AGA neonates (Ozyürek et al, 2006), reporting significantly higher platelet counts in the AGA group of 215 ± 6.0 x10^9/l (mean ± SE) compared with 182 ± 8.0 x10^9/l for the SGA group. A similar trend was apparent in the 40 SGA and 37 AGA neonates who were also sampled on day 7.

The data on mean platelet volume and birth weight is scarce, but this was studied in a subset of 52 neonates with birth weight >2000 grams and 15 neonates with birth weight <2000 grams (mean ± SD)(Arad et al, 1986). The mean platelet volumes were 8.45 ±1.1 fl (mean ± SD) and 8.67 ±0.82 fl respectively. Interestingly, the mean platelet volume increased over the first 20 days of life.

Source of sample

Capillary and venous platelet counts were compared in 95 healthy term neonates (Ozbek, Gürakan and Kayiran, 2000). The results for the venous samples were significantly higher at 213 ± 77 x10^9/l compared with 179 ± 68 x10^9/l for capillary samples taken simultaneously. The difference was most marked if there was also a high WBC. The process of obtaining a capillary sample may induce platelet activation with consequent aggregation and a reduction
in count. The same group subsequently published longitudinal data from a further 141 infants who were sampled at 7, 14, 21 or 28 days of age (Kayiran et al, 2003). At all these times platelet counts from capillary samples were significantly lower than the corresponding venous count.

Ethnicity

A Nigerian study reported 633 platelet counts, determined by phase microscopy, for 338 healthy term neonates on samples taken between 1 hour and 28 days (Effiong, Usanga and Mellits, 1976). There was a rise during the neonatal period from 207 ± 72 x10⁹/l (mean ± SD) during the first postnatal week to 282 ± 94 x10⁹/l for the remaining three weeks. A similar rise in platelet count was reported for neonates in Thailand (Sasanakul et al, 1993) and in the control group of the African study considering the impact of HIV (Mwinga et al, 2009). The mean values in all three studies were comparable to those reported for Caucasian populations.

Mean platelet volume

As well as bringing a welcome accuracy and reproducibility to the measurement of haematological parameters the use of automated counters also extended the range of parameters that could be measured routinely. One such parameter was the mean platelet volume (MPV), which became recognised as a predictor of the haemostatic potential of adult patients with severe thrombocytopenia (Bessman, Gilmer and Gardner, 1985). An increased MPV is usually associated with increased thrombopoiesis. The first paper on neonatal MPV determined by automated counting was published in 1982, reporting similar mean values for term and preterm neonates of 8.47 µ³ and 8.08 µ³ respectively (Kipper and Sieger, 1982). This was rapidly followed by a report aiming to provide neonatal reference ranges for MPV based on data from 155 neonates (Arad et al, 1986). In these analyses, the group was broken down by weight with 119 having birth weight >2000 grams and 36 with birth weight <2000 grams. Again, there was no significant difference in the mean MPV between the groups (8.67 +/- 1.03 µ³ and 8.89 +/- 1.10 , µ³ respectively), but the MPV rose in both with increasing postnatal age suggesting increased production of platelets in the early postnatal weeks.

MPV was analysed by the Intermountain Healthcare Hospitals group who found that the MPV remained fairly constant from 22 to 42 weeks, with a numerically small but statistically significant fall with increasing gestational age (Wiedmeier et al, 2009). The MPV rose over the first two postnatal weeks, with a subsequent slight fall to 28 days. As before, the
published data is presented graphically, giving an estimated MPV a birth of $7.5 \mu^3$ and a maximum 95\textsuperscript{th} percentile value of $12 \mu^3$ during the neonatal period.

\section*{2.8 Blood cell morphology in neonates}

There is very little published peer-reviewed literature on the morphological appearances of blood cells in apparently healthy neonates using conventional microscopy.

The red blood cell morphology of term and preterm infants has been compared with that of adults and children using interference-contrast microscopy (Holroyde, Oski and Gardner, 1969). Pits or craters were more common in the red blood cells of preterm and term infants compared with adults or older children, occurring in 47\% preterm and 24\% term infants compared with 3\% of adults. The frequency fell rapidly so that term infant values were similar to adults by 2 months of age. Preterm infants took longer to reach adult values. Interestingly, an inverse correlation was reported between birth weight and the number of pitted cells. It was suggested that the pitted red blood cells indicated hyposplenism – or more broadly reduced reticuloendothelial function.

A second study of neonatal red blood cell morphology described a technique using light microscopy and preparations fixed with 2\% glutaraldehyde which was used to compare the red blood cells of term and preterm neonates with those of adults (Zipursky et al, 1983). This reported an increased incidence of abnormal shapes in the neonates with more bowl shaped and irregular red blood cells and fewer disc shaped cells compared with adults. Zipursky identified a need for normative neonatal values to be determined.

Coulombel used phase contrast microscopy to describe two distinct reticulocyte shapes: a multilobular and a cup-shaped form (Coulombel, Tchernia and Mohandas, 1979). Sequential studies indicated that the multilobular form was immature compared with the mature cup-shaped form, and it was only seen in the peripheral circulation where there had been erythropoietic stress. Only the cup shaped form was seen in apparently healthy neonates. In clinical practice supravital staining with cresyl blue is used to stain reticulocytes. The results of manual reticulocyte counting were compared with results from an automated analyser (Advia 120) on samples from children >1 year old, term and preterm infants (Wiegand et al, 2004). The automated and manual results were similar for children >1 year old, but the automated analyser produced lower results than the manual for neonatal samples. This appeared to be because there was an increase in very mature reticulocytes in the neonatal samples which did not retain sufficient stain for automated detection.
Red blood cell changes associated with hyposplenism are also recognised as a feature of neonatal blood morphology with Howell-Jolly bodies, acanthocytes and spherocytes being present (Bain, 2002). Nucleated red blood cells are present in the first few days of life, but have disappeared by the fourth post-natal day in healthy term infants (Bain, 2002).

There is very little written about white blood cell morphology, but in a paper looking at lymphocyte vacuolation used as a screening tool for children with possible metabolic disease it is noted that monocytes may show non-pathological cytoplasmic vacuolation – especially if there has been a delay in making the film (Anderson et al, 2005).

Megakaryocytes are not commonly seen in the peripheral blood as they tend to be trapped in the fine capillary system due to their large size, and may just be visible as bare nuclei (Pedersen, 1978). Megakaryocytes are relatively more common in neonates compared with older children and adults, although they are also seen post-partum, post-operatively, in infection, inflammation, malignancy, disseminated intravascular coagulation and in myeloproliferative disorders (Pedersen and Petersen, 1980; Tinggaard Pedersen and Laursen, 1983; Murray and Roberts, 1995). There is an inverse relationship between post-conceptual age and the percentage of megakaryocyte progenitors in peripheral blood (Saxonhouse et al, 2004). The percentage of megakaryocyte progenitors also falls with increasing hypoxia (Saxonhouse et al, 2006).

A study of nine healthy term neonates described the presence of micromegakaryocytes (Levine et al, 1996). Over a third of all megakaryocytes had a diameter of <13 microns, and, in addition had reduced nuclear lobation despite being mature. A preliminary study of 100 healthy neonates measured the percentage of circulating megakaryoblasts in the peripheral blood, and reported that 0.02% cord blood nucleated cells were megakaryocytes (Bayliff et al, 2003).

Although there are a number of rare congenital syndromes associated with changes in platelet size and colour, there are no specific changes described in the healthy neonatal population.
2.9 Summary

Reference ranges for haematological parameters in neonates are established (Ablin et al, 1961; Matoth, Zaizov and Varsano, 1971; Manroe et al, 1979; Mouzinho et al, 1994) and historical standards are typically quoted in text books (Orkin and Nathan, 2009). More recently, reference ranges based on very large data sets using computerised records for the Intermountain Healthcare Hospitals in North America have been published and these represent the most comprehensive data to date (Christensen et al, 2008; Schmutz et al, 2008; Christensen et al, 2009; Wiedmeier et al, 2009; Jopling et al, 2009).

The literature on factors associated with changes in haematological parameters in neonates is small, but associations with birth weight, gestational age and postnatal age in particular are well recognised. Finally, the literature on blood cell morphology in neonates is very limited, although some specific features of blood cell morphology in neonates have been described.
Chapter 3  Children with Down Syndrome Study: methods

3.1  Introduction

The first part of this chapter describes the feasibility assessment and development work that was required to determine how a birth cohort of children with DS might be set up within the context of the National Health Service (NHS) in the UK. As there was no existing or historical cohort of this nature within the UK, a series of scoping exercises were required to understand the care pathways for a child with DS.

The latter part of this chapter describes the study design, setting, participants, sample collection, data collection, sample management, data input and statistical analysis of the CDSS with regard to this thesis. The complete study protocol is provided in Appendix 4.

A timeline of this work for the study is shown in Figure 3-1.

Figure 3-1. Timeline of the planning work involved in designing and establishing the CDSS.
3.2 Consultation phase

3.2.1 Consultations with professional stakeholders

Initial discussions about the proposal to study the haematology of children with DS from the neonatal stage onwards took place within a core group comprising of researchers from the Epidemiology and Cancer Statistics Group (ECSG; previously called the Epidemiology and Genetics Unit), and clinicians from the Leeds Teaching Hospitals (paediatric haematologists, a neonatologist and a community paediatrician). Once a decision had been made to develop a study, contact was made with two key stakeholder groups within the UK: the Down Syndrome Association (DSA) and the Down Syndrome Medical Interest Group (DSMIG). A series of meeting with the core group and with a medical representative from the DSMIG and a non-medical representative from the DSA then took place, with further email discussion between face to face meetings.

In addition to discussion with individuals from the DSA and the DSMIG the study plans were presented in an interactive session of the Annual Scientific Meeting of the DSMIG on 25/11/2005 attended predominantly by hospital and community paediatricians.

3.2.2 Consultation with clinicians

The next part of the consultation phase involved hospital paediatricians and neonatologists. The decision to work within the secondary care setting was predicated on the need to obtain blood samples from the neonatal period. The aim of these consultations was to understand key points in the practice of clinician involved in the management of babies and children with DS, and to canvas their willingness to participate. The emphasis was on understanding clinical practice and on listening to the experiences and observations of clinicians.

At the time of setting up the study, there were 12 hospitals offering neonatal care within the Yorkshire area. These are shown in Figure 3-2.
Figure 3-2. Map of the hospitals within the Yorkshire Neonatal Network.
The neonatal lead clinician at each of these hospitals was contacted, and a meeting requested with each of them. In addition the study proposals were presented at a Forum Meeting of the Yorkshire Neonatal Network on 11/10/2005 attended by the neonatal lead clinician from each hospital in Yorkshire. At each of these meetings the basic study aims were outlined and the following questions were asked:

1. How and when do you make and give the diagnosis of DS to a family?
2. Do you take a full blood count in the neonatal stage?
3. What is the subsequent care pathway of a child diagnosed with DS in your hospital?
4. When do they have blood tests taken in their early years?
5. Would you be interested in participating in a study of babies and children with DS?

3.2.3 Consultation with families

Once there was agreement over the design and wording of the study literature, the final phase of the consultation process began. This comprised of a series of informal meetings with families of children with DS to review and discuss the proposed study design and the literature.

Twenty families with a child with DS who lived within a 20 mile radius of the study centre, were contacted by letter explaining what was proposed and asking if they were willing to review the study literature. Of the twenty families invited to participate, ten families responded immediately to say that they would like to take part in the consultation. All of the ten families chose to meet in their homes, and each spent several hours reviewing the study documents and discussing the study.

Each meeting was a semi-structured interview which involved the following:

1. Explanation of the study rationale and study design
2. Family review of the study paperwork including the parent information sheets; parent consent forms and initial questionnaire;
3. Family reflection on their experience of the newborn stage, particularly relating to communication and diagnosis;
4. Family opinion on whether they would have participated in such a study had it existed when their child was born.

5. Family opportunity to suggest anything that they felt might be important or relevant that had been missed out;

3.3 Key points emerging from consultations

In addition to addressing the areas identified for discussion above, other strong themes emerged from the consultations. These themes, along with the response to the specific areas for discussion, are considered below and their impact on the study design is described.

3.3.1 Consultation with stakeholders

The main outcome of discussions with the DSA and the DSMIG was their active support, especially in attending meetings during the study design process and their subsequent promotion of the study. The stakeholders were particularly concerned with three areas of study design:

Broadening the scope of the study

Given the many important and unanswered questions relating to other aspects of health within this population, a decision was taken early on to broaden the scope of the study.

Preference for a phased study

It was very clear from discussions with the DSA and clinicians that they were concerned about how the newborn stage would be managed. For most families the diagnosis of DS is unexpected: of the estimated 765 live births in England and Wales in 2009, 717/765 (94%) were postnatal diagnoses (Morris, 2010). Individual families vary considerably in the responses they have to being told that their newborn baby has DS, but most experience varying degrees of associated negative feelings including grief, loss, anger, and sadness around the time of diagnosis (Hames, 1993; Skotko, 2005; Gath, 1985). Hence, it would have been much easier to set up a study in which families of a child with DS were approached when they were beyond this initial period. However, since a key aim was to collect biological samples in the neonatal period a way in which this could be done in an acceptable and sensitive manner needed to be negotiated.

After lengthy discussions it was decided that a phased process would be more appropriate. It was agreed that in the newborn period, when families were most vulnerable, the minimum amount of information and samples needed would be requested. However, the ‘door would
be left open’ so that we could subsequently go back to families to invite them to take part in the next part of the study when their child was a little older. The newborn stage is therefore essentially a cross-sectional study of neonates with DS, whilst the follow up group is a prospective cohort study of DS neonates.

Preference for local recruitment

Various possibilities for recruiting the children were considered. One option was to have one person, who would represent the study and was from outside the hospital setting, who would move from hospital to hospital to invite families to take part at the request of the local clinician. However, this idea was rejected as it was thought to be more appropriate for the study to be discussed with the family by the local clinical team with whom they already had a relationship. This avoided the family having to meet another new face, at a potentially difficult time, and would also mean that there was no time pressure surrounding the discussions about the study. The fact that there were likely to be a series of meetings between the clinician and family lent itself well to introducing the study, as there would be subsequent natural opportunities for the family to ask questions and discuss the study further.

3.3.2 Consultation with clinicians

The areas addressed with clinicians are all discussed in detail below.

Making and giving the diagnosis

Most babies are diagnosed with DS in the first few days after birth, and whilst they are still in hospital. The phenotypic features of DS may be recognised by a midwife, doctor or parent leading to further examination and cytogenetic testing to confirm the presence of a somatic trisomy 21. The stage at which a diagnosis of DS was likely to be made differed between the individual clinicians, with most preferring to have cytogenetic confirmation before making the diagnosis. All clinicians said that the child would stay in hospital pending the cytogenetic results - which usually took 48 hours - unless there were extenuating circumstances. Once the diagnosis of DS was clear this was usually discussed with the family by a senior clinician, often a Consultant Paediatrician. There then tended to be a series of meetings with the family on several occasions to discuss the diagnosis and its implications.

Although most clinicians usually waited for cytogenetic confirmation to give a definite diagnosis of DS, it was clear that in some cases a clinical diagnosis was made. It was therefore left to the clinician to decide at which stage they thought a diagnosis of DS was certain, and to decide when discussions about the study were appropriate.
Taking a full blood count in the neonatal period

It was normal practice in all twelve hospitals for neonates with a suspected diagnosis of DS to have blood taken for cytogenetic testing to confirm the diagnosis, even in cases where an antenatal diagnosis had already been made. Most clinicians agreed on the clinical features of a sick neonate that would prompt taking an additional blood sample for a full blood count. However, in the majority of babies these features are not present and consultation revealed a wide variation in practice as to whether a routine full blood count sample was be taken in an apparently well neonate. Practice varied between hospitals and also between clinicians in the same hospital. There was no hospital in which clinicians all routinely checked a full blood count in all neonates with DS. Only a minority of paediatricians were aware of transient myeloproliferative disorder (TMD) and its ramifications, and those that were tended to be the ones who had previously managed a child with the disease.

Care pathways following diagnosis

Arrangements for following up a child with DS tended to be informal. The initial care would be led by the consultant on call, who was variably a neonatologist or general paediatrician depending on the composition of consultants on the on call rota. This consultant might continue to manage the child throughout their childhood, or might refer the child on to a colleague during the diagnostic process, on discharge from hospital, during the first or second year of life, or once they had successfully come through management of a particular clinical problem. The consultant colleague then responsible for care was variably a hospital or community paediatrician who might or might not have a special interest in neurodisability or Downs syndrome. The different pathways are described in Figure 3-3.
Taking blood tests during the early years

The DSMIG provide guidelines for the management of children with DS, available on their website and also in specific inserts which fit in the Personal Child Healthcare Record (PCHR) – the PCHR is a book given to a child’s parents/carers on their birth which is used to record growth, development, and use of health services. The DSMIG recommends that the inserts are given to every family once a diagnosis of DS is made (Appendix 5). The guidelines recommend that a yearly blood sample is taken for thyroid function testing in areas where finger-prick testing is available. This applies to most hospitals in the region. Where finger-prick testing is not possible a minimum schedule of blood tests at 12 months, 3-3½ years and 5 years is recommended.

Although all clinicians in the twelve hospitals were aware of the DSMIG inserts provided for the PCHR, awareness of the content of the guidelines provided in these was variable and adherence to them appeared to be low. Prior to the consultation with the clinicians it had been supposed that most children would be having routine annual blood tests, when an extra sample could be taken for the study. It was decided that the aim would still be to take an opportunistic sample when the ‘routine’ bloods were taken, but it was recognised that these may be less frequent than at first expected.
Willingness to participate in the proposed study

All the clinicians across the 12 hospitals were keen to participate, and agreed to assign a lead clinician for each hospital. In one Trust where the study was presented at a joint meeting of two hospitals in the Trust some of the clinicians from one of the hospitals had already been taking part. At this meeting one of the senior paediatricians commented that he did not like studies as a rule, but had found this one easy to discuss and that the families had found it a positive support. The outcome of the contact with each hospital is outlined in Table 3-1.

Table 3-1. Willingness of clinicians in the Yorkshire Neonatal Network to participate.

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Initial response</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airedale General Hospital</td>
<td>Invitation to present the study at the paediatric consultants’ meeting on 05/12/05</td>
<td>Departmental agreement to take part at the end of the presentation and study lead appointed</td>
</tr>
<tr>
<td>Bradford Royal Infirmary</td>
<td>Invitation to present the study to the neonatal lead clinician and to the lead for DS on 21/9/05</td>
<td>Agreement to participate after further email discussion and study lead appointed</td>
</tr>
<tr>
<td>Calderdale Royal Hospital</td>
<td>Immediate agreement to participate from the Neonatal Lead Clinician</td>
<td>Study lead agreed in advance of presentation. The earliest date that could be set to present the study to the department was 18/06/06.</td>
</tr>
<tr>
<td>Dewsbury and District Hospital</td>
<td>Invitation to present the study to the paediatric consultants on 18/11/05</td>
<td>Departmental agreement to take part at the end of the presentation and study lead appointed</td>
</tr>
<tr>
<td>Harrogate District General Hospital</td>
<td>Invitation to present the study to the paediatric departmental meeting on 3/11/05</td>
<td>Departmental agreement to take part at the end of the presentation and study lead appointed</td>
</tr>
<tr>
<td>Huddersfield Royal Infirmary</td>
<td>Invitation to meet the neurodisability lead on 26/09/05</td>
<td>Agreement to take part after discussion with paediatric consultant colleagues and study lead appointed. Subsequent study presentation to joint meeting with Calderdale Royal Hospital on 18/06/06</td>
</tr>
<tr>
<td>Hull Royal Infirmary</td>
<td>Invitation to present the study to all the paediatric consultants on 7/12/05</td>
<td>Departmental agreement to take part at the end of the presentation and study lead appointed</td>
</tr>
<tr>
<td>Leeds General Infirmary</td>
<td>Invitation to present the study to the neonatal departmental meeting on 21/11/05</td>
<td>Departmental agreement to take part at the end of the presentation and study lead appointed. Subsequent meeting also with the antenatal screening coordinator on 21/11/05</td>
</tr>
<tr>
<td>Pontefract General Infirmary</td>
<td>Invitation to present the study to the paediatric departmental meeting on 07/02/06</td>
<td>Departmental agreement to take part at the end of the presentation and study lead appointed</td>
</tr>
<tr>
<td>St James’s University Hospital</td>
<td>Invitation to present the study to the neonatal departmental meeting on 7/11/05</td>
<td>Departmental agreement to take part at the end of the presentation and study lead appointed</td>
</tr>
<tr>
<td>Scarborough District General Hospital</td>
<td>Invitation to meet the Neonatal Lead Clinician on 28/11/05</td>
<td>Agreement to take part at the end the meeting and study lead appointed</td>
</tr>
<tr>
<td>York District General Hospital</td>
<td>Invitation to present the study to the departmental meeting on 11/11/05</td>
<td>Departmental agreement to take part at the end of the presentation and study lead appointed</td>
</tr>
</tbody>
</table>
Study co-ordination

Many clinicians were concerned about the workload that the study might entail for them. Although the initial introduction to the study occurred in the clinical setting, the study was set up so that subsequent communication occurred directly between the families and the study centre. A minimum amount of information was requested from the clinician at entry, primarily to ensure the information needed to identify the child, parents, referring clinician and general practitioner was correctly recorded. Thereafter, the families interact directly with the study centre. Families choose the pace at which they take part in the study allowing for the differences in adjustments to the diagnosis, and for the practicalities of managing a newborn – especially one who may have pressing medical needs. Similarly, blood sampling kits for taking the samples during follow up are sent directly to the families who are then responsible for taking the kit to the relevant appointment.

Communication of results

Many of the clinicians expressed a wish for the results of the neonatal full blood count analysis to be fed back to them. If a child was unwell and a full blood count was indicated clinically then this would be done immediately anyway and processed independently of the study in the hospital laboratory as usual. However, clinicians said they would find it useful to identify apparently well neonates who might have a haematological abnormality, especially TMD. In this case the full blood count result was not urgent, although it was important and the consensus was that it would be helpful for the neonatal full blood count result to be fed back to the clinician. It was agreed that results of the subsequent blood tests would only be fed back if there was a concern from the study centre.

3.3.3 Consultation with families

This phase of the consultation was very significant as, although representatives from the DSA and the DSMIG had been involved from the start, it marked the first time that families had been directly approached. In the course of developing the study a number of different people had expressed their opinion that, however important such a study might be, it would be practically too difficult to achieve given the sensitivities in approaching families with a newborn child diagnosed with DS. It was therefore very encouraging that the family consultation found overwhelming support for the study from families who had been in such a position very recently themselves. Several families expressed a view that we could ask for participation in the follow up right from the start, although the majority view was that the staged process was sensible. Minor amendments to the wording of the study literature were made.
3.3.4 Summary of changes to study design following consultation

1. The scope of the study was broadened to include other aspects of health;

2. Participation was phased with newborn and follow up stages;

3. Recruitment would be by a clinician already known to the family;

4. Cytogenetic confirmation of DS was not required if a firm clinical diagnosis had been made;

5. The amount of paperwork required from the local clinician would be kept to a minimum;

6. The result of neonatal full blood count would be fed back to the clinician.
3.4 Study design

3.4.1 Study type

The CDSS is an observational birth cohort with two parts: a cross-sectional study during the neonatal period and a longitudinal study during the early years.

3.4.2 Pilot phase

The pilot phase ran from May 2006 to December 2006 inclusive and took place primarily within the Yorkshire Neonatal Network. The aim of this phase was to test the study design in a limited setting where there were existing links with the clinicians and hospitals. The progress of the pilot phase up to 31/12/2006 was reviewed at a core group meeting on 03/01/2007.

3.4.3 Ethical permission

Ethical approval for the study was granted by the Research Ethics Committee for Wales in March 2006: 06/MRE09/16. This approval included site specific exemption.

3.4.4 UKCRN Portfolio status

The UK Clinical Research Network (UKCRN) is part of the National Institute for Health Research, which was created in 2006 as part of a major restructuring exercise aimed at developing and streamlining clinical research. A central strategy is the recognition of well-designed studies and the provision of an infrastructure to support these within the context of the local National Health Service. The CDSS was registered as an UKCRN Portfolio study in April 2008 (Study ID 3088).

3.5 Study setting

Within the NHS managed clinical networks for neonatal care were established in 2004 following the model of managed networks for cancer. Each Neonatal Network ([www.bapm.org/networks_info/](http://www.bapm.org/networks_info/)) contains around 8-12 hospitals. The initial study area was restricted to the 5 Neonatal Network areas most accessible from Leeds and York. During the pilot phase the Trent Neonatal Network asked to participate in the study. The CDSS was piloted in the Yorkshire Neonatal Network before being rolled out to five more Neonatal Networks: Northern, Greater Manchester, Cheshire and Merseyside, North Trent and Trent. The geographical target area is show in Figure 3-4.
Figure 3-4. Map of the target area for the CDSS.
Although most babies are born in a hospital maternity unit with an on-site neonatal service, this is not always the case. There are some stand-alone maternity units which rely on neonatal support from another site – Wansbeck is an example of this, being supported by the Royal Victoria Infirmary in Newcastle. In this case the stand-alone maternity unit was aware of the study taking place in the linked neonatal department. Additionally, some babies are born at home. Once the possibility of a diagnosis of DS was raised for a baby delivered at home they would be referred to the local neonatal service. During the course of the study maternity units at Bishop Auckland, Huddersfield Royal Infirmary and at Trafford General Hospital closed down due to NHS reorganisation.

In order to set the study up in an individual hospital contact was made with a clinician in each hospital, introducing the study and requesting an opportunity to present the study, usually to a paediatric departmental meeting. Once a centre had confirmed that they wished to participate and a study lead had been appointed the relevant approval was sought from the local research and development department.

3.6 Participants

All children born with DS from 1st May 2006 onwards were eligible to take part in the newborn stage. A minority of babies with DS are diagnosed prenatally - around 6% in 2009. Families with a prenatal diagnosis of DS were not approached before birth.

3.6.1 Identification of participants: newborn stage

Identification of cases, approach and recruitment into the newborn stage typically took place after birth and prior to hospital discharge. A supply of CDSS newborn entry packs was provided to each participating hospital to be kept readily accessible in the appropriate clinical area. The contents of the CDSS newborn entry packs are shown in appendix 5. When a newborn was identified as probably having a diagnosis of DS, and therefore being eligible for the study, a newborn entry pack was opened and an alert card completed. The alert cards were prepaid cards addressed to the ECSG. They did not have any personal information on, but functioned as a means to improve communication and thus recruitment rates, and also as a possible means of estimating participation rates.

When appropriate the study was discussed with the family by a member of the clinical team looking after the child. This was not necessarily the local study lead, but was a health professional closely involved in the care of the child. At this stage families were given written information about the study provided in the CDSS newborn entry pack. This written
As well as explaining why, how and where the study was being done, the information sheet contained contact details should families have any queries. These included a “freephone” number, an email address, the study website, and also a postal address.

On the ‘parental consent form – newborn’ families were asked for permission to allow:

1. A full blood count sample from their child to be sent for full blood count analysis and storage;

2. Access to the medical records of the mother that pertained to the pregnancy and birth of the index child;

3. Their GP and Health Visitor to be informed that they are taking part in the study

4. The study centre to approach the family at a later date in order to discuss the next stage of the study.

Families could opt out of any of the above and still participate. The consent form was provided in triplicate - one copy for parents, one copy for the medical notes, and one copy to be returned to ECSG in a prepaid addressed envelope provided in the pack.

The pathway for the newborn stage is described in Figure 3-5.

**Figure 3-5. Initial pathway into the CDSS.**
3.6.2 Action taken after initial recruitment

Once the ‘parental consent form – newborn’ had been received at the study centre a letter was sent to the hospital consultant to confirm that the child had been entered into the study. An information sheet about the CDSS and a copy of the full blood count result, where relevant, were also enclosed. A letter was also sent to the child’s general practitioner to inform them that the child had been entered into the study, along with an information sheet regarding the CDSS and a copy of the newborn consent form. A letter was also sent to each family on receipt of the ‘parental consent form – newborn’ to thank them for their involvement. The pathway following initial recruitment is shown in Figure 3-6.

During the pilot phase of the study contact was made with the general practitioner or hospital clinician, when the child was around three months of age, to confirm that it was still appropriate to approach the family again. Once confirmation was received the family were sent: a letter inviting them to join the follow up, an information sheet with further information about the study, and a basic questionnaire. Following review of the pilot phase this process was amended so that families were sent a prepaid addressed card for them to return if they would like further information about the follow up stage as part of the initial letter they received thanking them for their participation in the newborn phase so that those who wished could join straight away. If a card had not been received from the family then they would be contacted at the four month stage.
3.6.3 Identification of participants: follow up

Once a request for further information about the follow up study was received, the family were sent a ‘study information leaflet – follow up’ and a ‘parental consent form – follow up’ along with an explanatory letter. All the literature for the CDSS is publicly available on the website: www.cdss.org.uk.

On the ‘parental consent form – follow up’ families were asked for permission to allow:

1. Questionnaires about the child’s health and background to be sent out;

2. Access to the child’s hospital records;

3. Access to the child’s records held by the general practitioner and by the health visitor;

4. Additional blood samples to be taken when the child was having a routine blood test;

5. A buccal swab to be taken.

Families were asked to complete the consent form, indicating which parts of the study they would like to take part in, and then to return the form in the prepaid addressed envelope provided.
3.7 Sample collection

A blood sample kit was provided in the CDSS newborn entry pack. This comprised of an EDTA tube, a form for the laboratory, and a prepaid addressed envelope so that it could be sent directly to the designated haematology laboratory for analysis. The form accompanying the sample requested information about the child’s full name, date of birth, gender, hospital number, name of the sending hospital, name of clinician taking the sample, source of sample (venous or capillary), the date the sample was taken and the time at which the sample was taken. Where possible it was requested that a fresh full blood count sample was taken in the EDTA tube provided. If permission to take a fresh sample was not granted, but if there was a recent sample in the local hospital laboratory then this was retrieved and sent in the envelope provided.

Subsequent blood samples were taken during the follow up stage when the child was having routine blood tests. The DSMIG guidelines recommend that blood tests are taken annually to monitor thyroid function. The packs for blood sample collection were sent directly to families. The packs contain an EDTA tube, a form and a pre-paid addressed envelope for return to the designated haematology laboratory. Families were asked to take the pack to the clinic when a blood test was due and to then post it directly to the haematology laboratory in the pre-paid addressed envelope provided.

3.8 Data collection

3.8.1 Hospital referral form

Socio-demographic information about children in the newborn stage was initially obtained from the ‘hospital referral form’ contained within the newborn entry pack. Details requested included:

1. Child’s full name, date of birth, sex, NHS number and hospital number;
2. Mother’s full name and hospital number;
3. Contact details for the child’s GP and paediatrician;

This is the only paperwork that the clinicians were required to complete, and from this point on the study centre liaised directly with families. A copy of the hospital referral form is provided in Appendix 6.
3.8.2 Initial questionnaire

A questionnaire was sent to the family soon after recruitment. This requested the following information:

1. Address, postcode, telephone numbers and email address;
2. Child’s full name, date of birth, sex, NHS number;
3. Mother and father’s full names, dates of birth and NHS numbers;
4. Contact details for the child’s GP and paediatrician;
5. Details of the hospital attended for maternity care, and maternity number;
6. Details of the hospital where the child was born, if different;
7. Child’s birth weight, gestational age, mode of feeding and details of any admission to a Special Care Baby Unit or Neonatal Intensive Care Unit.

In addition, there was a section for free text. A key function of this was to ensure that all the family and medical contact details are up to date. This latter is particularly important where the child was under the care of clinicians in a variety of health care settings so that when the abstraction of medical records starts all the relevant records will be accessed.

3.8.3 Abstraction from medical records

More detailed information will be abstracted from medical records, which include the obstetric, neonatal, general practitioner and health visitor records. This will include socio-demographic data as well as information on clinical status. Studies of mortality in children with DS have shown that approximately 5-10% will die in the first year of life (Leonard et al, 2000; Frid et al, 2004). With this in mind the process of accessing hospital medical records in order to abstract medical information has already been started by ECSG staff. This is also important as it can be difficult to retrieve hospital obstetric and neonatal records once an admission has been completed – and indeed in some hospitals records are being electronically archived soon after discharge if they are no longer considered as being ‘active’. The ECSG has experience of abstraction from other studies and has developed abstraction forms specifically designed for this setting.

General practitioner and health visitor records are not archived in the same way as hospital records. In order to make this process most efficient, this part of the abstraction will start once the children are 5 years old.
3.9 Sample management

3.9.1 Processing of neonatal blood samples

Neonatal blood samples were initially analysed at the haematology laboratory in St James’ University Hospital (SJUH), using an Advia 2120. In October 2006 the location changed to the haematology laboratory at York Hospital (YH) where the samples were processed on a Sysmex XE2100. A standard operating procedure for processing the samples was followed by the haematology laboratory (Appendix 7). Both the haematology laboratories have full CPA accreditation (Appendix 2) and complete regular external quality assessment, co-coordinated by the UK NEQAS Scheme for Haematology (Appendix 3).

As part of the standard operating procedure three blood films were made on all neonatal blood samples upon arrival in the haematology laboratory, initially at SJUH, and then from October 2006 at York Hospital. One of these slides was stained and two slides were left unstained. The haematology laboratory at SJUH uses a Leishman stain, and a May-Grünwald-Giemsa stain is used at York Hospital (Lewis et al, 2006). Each stained slide was reviewed using the morphology review tool designed for the CDSS.

After collection from the haematology laboratory, the remaining EDTA sample is stored at -80°C and the three blood film slides made in the haematology laboratory were stored at room temperature in the University of York.

3.9.2 Processing of follow up blood samples

Follow up blood samples are all sent to the haematology laboratory at YH where they were processed on a Sysmex XE2100. Again, three slides are made for morphological review. The remaining EDTA sample and the three blood film slides made in the haematology laboratory were then collected from the haematology laboratory and stored at -80°C in the University of York.

3.10 Data management

3.10.1 Data management

All children enrolled in the study have been given a unique identifier. Data and information collected throughout the study along with results of sample analysis is held in a secure database. All questionnaires and additional paperwork is stored appropriately in locked cabinets for future use.
3.10.2 Data entry

A database was designed specifically for the CDSS using a Microsoft Access interface and SQL server data storage. More recently, a web-based interface was developed by ECSG staff. The database records and links all the socio-demographic and clinical data for a study participant with information from their biological samples. Data entry was performed by different members of the ESCG staff.

3.11 Dissemination of information

3.11.1 Feedback to clinicians

The only result to be fed back to the clinician was the result of the neonatal full blood count analysis. Clinicians were not routinely informed about subsequent results unless these were suggestive of a problem that might require clinical management in which case the result was discussed with a paediatric haematologist at the Regional Centre for Paediatric Haematology at Leeds General Infirmary and appropriate action taken. In order to standardise the procedure for referral to the Regional Centre for Paediatric Haematology a list of triggers for referral for a clinical opinion was drawn up (Appendix 8). In cases where results met these criteria the hospital clinician was contacted and informed so that they could take things forward as clinically appropriate. Regular progress reports about the study were produced for the lead clinicians in each hospital, and these were disseminated by email.

3.11.2 Website and family forum

Individual results were not fed back to families. Instead, information about the study progress and about presentations or publications was made publically available on the study website (www.cdss.org.uk). In addition, families taking part in the CDSS were also able to access a password protected message forum.

3.12 Statistical analysis

3.12.1 Neonatal full blood count analysis

Analysis was restricted to full blood count results from children born between 1st May 2006 and 1st October 2011 with a sample taken at ≤ 28 days of age. Where multiple results were available for the same child only the first set of results meeting these criteria was included. The full blood count parameters studied were haemoglobin, haematocrit, red cell count, mean cell haemoglobin, mean red cell volume, nucleated red blood cell count, white blood cell count, neutrophil, lymphocyte, monocyte, eosinophil and basophil count, platelet count and
mean platelet volume. Mean (95% CI) and ranges (5, 95% percentiles) were determined for each parameter.

Regression analyses examining potential associations with birth weight, gestational age, postnatal age and sampling to processing interval on each of the full blood count parameters were assessed using a linear regression model (the Genmod procedure, The SAS System v9.2, SAS Institute, Cary, NC, USA) with non-linear effects assessed by the addition of a centred quadratic term for the factor under investigation to the model. Associations with birth weight and gestational age were assessed only in children sampled in the first seven days of life, adjusting for age at sampling. The effect of the sampling to processing interval, and also the effect of age at sampling were assessed by including all children sampled within the first 28 days of life. Trends in each parameter as the days between venepuncture and analysis of the sample increased were assessed graphically and using a univariable linear regression model (the Genmod procedure, The SAS System v9.2, SAS Institute, Cary, NC, USA).

3.12.2 Neonatal morphological analysis

Morphological analysis was restricted to blood films from children born between 1\textsuperscript{st} May 2006 and 1\textsuperscript{st} October 2011 with a sample taken at ≤ 28 days of age. Again, where there were multiple slides from samples taken from the same child only the first set of results was included. The morphology review tool developed for the CDSS was used. Regressions examining the presence or absence of particular anomalies used logistic regression – this approach was also used when assessing the proportions of cells with a particular abnormality. Regressions using the numbers of a particular type of cell as the outcome (for example, the number of hypergranular neutrophils) were done with Poisson regression using the number of cells at risk (in this example, the total number of neutrophils) as an offset.

3.12.3 Full blood count analysis beyond the neonatal stage

Analysis was restricted to cases where there was a full blood count result from a child born between 1\textsuperscript{st} May 2006 and 1\textsuperscript{st} October 2011 and with a sample taken within 3 months of each birthday including their birth and up to their 4\textsuperscript{th} birthday. Where multiple results for the same child were available only the set of results closest to their birthday was included. The full blood count parameters studied were haemoglobin, haematocrit, red cell count, mean cell haemoglobin, mean red cell volume, nucleated red blood cell count, white blood cell count, neutrophil, lymphocyte, monocyte, eosinophil and basophil count, platelet count and mean platelet volume. Mean (95% CI) and ranges (5, 95% percentiles) were determined for each parameter.
Chapter 4 Development of the morphology reporting tool

4.1 Introduction

This chapter describes the development of the morphology reporting tool used in the CDSS. This was a complex and lengthy process. The chapter begins by reviewing the historical development of blood cell morphology and current attempts to standardise it. The initial approach taken for morphology review was not successful, necessitating the development, validation and implementation of a new morphology reporting tool which would allow credible analysis.

4.2 Historical development

The appearance of blood cells was first described by Leeuwenhoek in 1674 (Leeuwenhoek, 1674), although it was another 200 years before the successful development of dyes enabling the finer details of cell structure to be seen (Ehrlich, 1877). An immediate outcome of this staining was the recognition that white blood cells could be further differentiated into neutrophils, basophils, eosinophils, monocytes and lymphocytes – a classification that still remains in use.

The next advance was the development of manual methods for counting red blood cells, white blood cells, and platelets. These techniques would be performed in tandem with microscopic examination of the blood film to determine the size and shape of red blood cells and platelets along with a differential white blood cell count. Although the processes were refined and improved throughout the first half of the 20th century, variability between operators remained a concern until the laboratory process was revolutionised by the development of the first automated blood cell analyser in 1956. This was based on the principle of single channel impedance counting and was able to measure indices directly (Coulter, 1956). This breakthrough was followed by increasingly sophisticated multi-parameter analysers able to give information about all three cell types (red and white blood cells, as well as platelets) and to provide a white blood cell differential. Thus, manual techniques gave way to modern analysers which are demonstrably precise, accurate and have the advantage of being able to turn over a large number of samples rapidly and with little manual input (Pierre, 2002).

Initially the results from the automated analyser were complemented by results from microscopy examination. However, microscopy examination can be a time consuming process. The automated analyser is recognisably superior in cell counting and in obtaining a white blood cell differential, at least in cases with typical white cell structure. Morphological
examination is therefore now reserved for specific cases where the automated analyser alone is inadequate. There are three main situations in which this is the case:

1. A problem with the sample – for example, platelet clumping, making the platelet count unreliable;
2. Abnormal white cell morphology – making it impossible for the analyser to generate a differential;
3. Numerical parameters outside accepted limits set by the local laboratory – each hospital sets ‘rules’ which indicate a need for a blood film to be made and examined. These ‘rules’ tend to be local, although there is a recommendation and protocol for local validation from the International Consensus Group for Haematology Review (Barnes et al, 2005; Cui et al, 2010; Woo et al, 2010).

In the United Kingdom the National External Quality Assessment Service (NEQAS; Appendix 2) has included blood film morphology in its assessment since 1968. A Selection Committee chooses blood slides to be sent out for assessment. Considerable effort is taken to ensure that the slides are spread, stained and fixed according to strict protocols in order to maintain batch integrity. The slides are chosen for slide quality, as well as for interest and range of conditions. Currently around six hundred laboratories participate in the scheme each receiving two slides on eight occasions over the course of a year. Minimal clinical information is provided – typically only the age and gender of the patient, along with the haemoglobin and white cell count (not the platelet count). Participants complete a report selecting a maximum of five coded comments from the sheet provided by NEQAS. This simply records the presence of abnormal features; no attempt is made to grade the frequency of these. In contrast to other NEQAS schemes, the performance of individual laboratories is not scored and it is recognised that this scheme ‘probably has not been standardised’ (personal communication; B de la Salle). Rather, the scheme is provided as an educational tool to support laboratories in their in house training. More recently, NEQAS has piloted a scheme using digital technology (Brereton et al, 2008).

There are a few earlier isolated reports of attempts to apply quality control to morphology (Rajamäki, 1979; Rajamäki, 1980), but it has mostly remained uncontrolled. In the earlier of these two reports 24 blood films were sent out to 206 different laboratories over a 4 year period. None of the films had either a high white cell count or abnormal white blood cells. There was good inter-laboratory consistency for segmented neutrophils, lymphocytes, basophils and eosinophils, while band neutrophils and monocyte counts showed more variation. It was suggested that the distinction between a band neutrophil and a segmented
neutrophil varied between laboratories and monocytes might be confused with lymphocytes in some cases – this problem did not affect the lymphocyte count as much as the number of lymphocytes was much greater. Problems in differentiating between band and segmented neutrophils and between normal and reactive lymphocytes are well recognised (Koepke, 1977). Overall, the author concluded that inter-laboratory assessment was a useful tool to improve the quality control of morphology. Following this a Finnish inter-laboratory proficiency testing programme in haematological morphology was set up (Rajamäki, 1980). The two main components of this were the use of a standardized form to collect results and the creation of a reference board who decided what was referred to as the “morphological ‘truth’”. One of the important points to emerge from this national process was the decision that a consensus of ≥80% with the reference board was the appropriate cut-off for inter-laboratory reliability.

The Finnish work highlights the potential for inter-laboratory and inter-observer variability in the calculation of the manual white blood cell differential. Nonetheless when automated differential counting was first introduced the manual differential count was considered as the gold standard against which the automated count must be viewed (Simmons and Elbert, 1975; Bain et al, 1980), and, despite the recognition of its weaknesses, performance against the manual count is still a key element in the assessment of new automated differential counting techniques (van de Geijn et al, 2011). Lewis turns this idea around, making the point that the automated count can be used to assess the reliability of the manual differential (Lewis, 1990). Automated differentials are based on the assessment of much greater numbers of white blood cells than manual differential counts which are typically based on 100 or 200 cells. Even the very earliest automated counters generated a differential from counting ~10000 white blood cells. This means that there is a greater likelihood of sampling bias in a manual count. The way that the film is stained and spread can then become critical in the reliability of the manual count.

The importance of the morphological assessment of blood cells, and the inherent problems of standardisation have since led to an internationally focussed drive to apply external quality assessment processes to morphological assessment of blood films (Vives Corrons et al, 2006; Gutiérrez et al, 2008). In a similar manner to the Finnish effort to apply external quality control to the assessment of the white blood cell differential count, the Spanish Haematology and Haemotherapy Association describe an external quality assessment scheme which focussed on the ability of laboratories to recognise significant morphological features for all cell lines (Gutiérrez et al, 2008). Over a 6 year period 25 blood films were sent to 604 participants who were required to choose the four most important morphological features on
each film from a given list. In addition to the blood film, they were provided with basic clinical details. Again, the results were compared with an independent assessment of what the “true” answer was. Interestingly, one of the most significant findings was that the most reliable results came from laboratories attached to hospitals, which consistently outperformed those from stand-alone laboratories.

An important contribution has also been made recently by the European Leukaemia Net Morphology Faculty which has produced a consensus statement, based on the reports of 28 expert morphologists, and a freely available web-based glossary for the identification of haematopoietic cells in the context of malignancy (Zini et al, 2010). However, at the current time there is no standard means of reporting blood cell morphology, particularly benign haematology, and so one had to be developed for the purposes of the study.
4.3 Initial approach to morphology review

4.3.1 Development of the morphology proforma

In the absence of a recognised tool for reporting blood cell morphology one needed to be developed specifically for the study. As a starting point the cytomorphology lead for the current MRC UKALL study was contacted and blood film review proformas used for the Medical Research Council UK ALL, AML, MDS and Interfant trials were obtained (Jenny Britton; personal communication). These proformas were themselves based on the landmark paper from the French-American-British collaborative group proposing consensus criteria for the classification of acute leukaemias following review of over 200 blood slides (Bennett et al, 1976). They all consisted of a small section with some basic clinical details on, followed by a space for the reviewer to add their manual white blood cell differential count and ending with a section for the reviewer to note specifically whether certain morphological features were present of absent – and if present to grade them.

A proforma for the study was drawn up, based on this same model, and in discussion with a small group of experienced consultant paediatric haematologists. It was agreed that the basic clinical details that would be provided would be the child’s gender, ethnic origin, gestational age, date of birth, age when sample taken and source of sample. The automated full blood count was to be provided. The reviewer was required to complete two main tasks: firstly, to determine the white blood cell differential count; and, secondly, to assess specific morphological features for all cell lines. During the initial morphology review there were various versions of the proforma, each with minor amendments. The proforma used for the majority of the reviews was version 3, which is shown in Figure 4-1. The amendments made to the different versions included the following:

1. The addition of specific morphological abnormalities for comment. The initial list of abnormal morphological features was drawn up from the form used for the myelodysplasia review and also from articles reporting on morphological features found in blood films for babies with DS reviewed in the previous chapter. After some of the early films had been reviewed, other morphological abnormalities were noted and were added to the form. These included: eosinophil vacuolation, basophil vacuolation, lymphocyte vacuolation, monocytes with stellate nuclei,

2. Changes to the grading system used to indicate the severity of a particular feature. Initially, the reviewer was asked to decide the degree of a particular feature and to assign it as absent; mild (1-10%); moderate (11-20%); or severe (>21%). However, some reviewers expressed concern over the use of percentages to describe the
degree and incidence of dysplasia. This generated lively debate. It was suggested that the categories could be simplified to occasional=<10%, moderate=10-50% and plentiful=>50% to try to make it less subjective. However, the final consensus was that percentages should be abandoned in favour of purely descriptive terms.

3. Some reviewers tended to identify the haematopoietic lineage of blasts, where seen and where possible, and so this option was included.

4. It was noted that the platelets were frequently commented on as being ‘pale’ or ‘ghostly’ and so a questions were added specifically asking whether the platelet colour was felt to be abnormal, and if so whether the platelets appeared paler than normal.

5. Finally, in the light of concerns about discrepancies between the manual and automated counts a question was introduced specifically asking if the two were felt to be congruous.
Figure 4-1. Proforma used for initial morphology reviews.

**BLOOD FILM REVIEW**

- For the Northern Down's Syndrome Study and the Oxford based Neonatal Down syndrome study

Sex: 
Ethnic origin: Venous or capillary ?
DOB:/dd/mm/ Gestaional age: weeks Age at testing: weeks days

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<td>RCC</td>
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<td>NRBC</td>
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<td>Blasts</td>
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<th>MANUAL DIFFERENTIAL COUNT</th>
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<td>DEGREE OF DYSPLASIA</td>
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<td>Azurophilic granules</td>
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<td>RED CELLS</td>
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<td>Megakaryocyte fragments</td>
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**OTHER COMMENTS**

________________________________________________________________________

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________________________________________________________________________

Slide reviewed by ____________________________________________________________________________

Date _______________________________________________________________________________________

Version 3, 3rd July 2006
4.3.2 Inter-operator variability in the differential white blood cell count

The interim analysis considered the reviews made on 13 slides. Eleven of these had been reviewed by all three reviewers (slides 005D, 022D, 030D, 033D, 038D, 039D, 046D, 049D, 053D, 079D, 095D) and a further 2 slides had been reviewed by two reviewers (slides 007D, 010D). Preliminary analysis of the differential white blood cell count indicated that most results were in agreement using the standard from the Finnish inter-laboratory proficiency testing programme in haematological morphology (Rajamäki, 1979), which requires results to be ≥80% of the ‘truth’, here taken as the mean value for the three reviewers. All agreed on three films where a differential count was not possible – this was important as it is likely that more mistakes might be made trying to do a white blood cell differential on a poor film where many cells are disrupted.

Where there were obvious discrepancies between the three reviewers these were as follows. Comparison is with the other two reviewers:

1. Reviewer 1:
   a. Lower neutrophil counts (slides 038D, 046D, 049D)
   b. Higher lymphocyte counts (slides 022D, 030D, 039D, 046D, 049D, 053D)
   c. Lower monocyte counts (slides 010D, 022D, 049D, 053D)

2. Reviewer 2:
   a. Lower monocytes (slides 039D, 046D)
   b. Reported blasts, but did not designate a sub-type in contrast to at least one other reviewer (slides 022D, 030D, 039D, 046D, 049D, 053D, 079D)

3. Reviewer 3:
   a. Lower lymphocyte counts (slides 005D, 030D, 038D, 046D)
   b. Higher monocyte counts (slides 005D, 039D)
   c. Higher myelocyte counts (slides 038D, 079D)
   d. Metamyelocyte count >50% of the others at 7x 10^9/l (slide 005D)
   e. Eosinophil count >50% of the others at 13x 10^9/l (slide 030D)
   f. Nucleated red blood cells >35% of the others at 212x 10^9/l (slide 038D)
   g. Higher blast count (slide 038D, 053D)
   h. Ascribed a blast type in 3 films where neither of the others did (slides 038D, 046D, 049D, 053D), and in 4 films where only one other did (slides 022D, 030D, 039D, 079D)
   i. Isolated abnormality where a much higher blast count was recorded whilst reviewer 1 recorded a much higher monocyte count (slide 038D)
Figure 4-2. Deviation of the manual from the automated differential white blood cell count.

In order to try to understand these discrepancies and to determine whether they were due to systematic or to random errors the mean percentage for each differential white blood cell type was calculated for each reviewer and was compared with the mean percentage derived from the automated differential count. The results are presented in Figure 4-2 as the deviation from the automated count.

The automated count usually reported neutrophils, without distinguishing between neutrophil precursors such as myelocytes, metamyelocytes or promyelocytes. Three of the counts were processed on the Sysmex XE-2100 which does report the immature granulocyte count, although there was only one case in which cells were recorded in this category (corresponding to slide 038D). The mean counts were adjusted to give the total for the combined counts for mature and precursor neutrophils. The deviation of the manual neutrophil values from the automated value consequently reduces, as seen in the amended comparisons shown in Figure 4-3.
This confirms the initial impression that there were important discrepancies between reviewers. Reviewer 1 reports a lower neutrophil count when compared with the automated count. Reviewer 3 reports a lower lymphocyte count and a higher blast count when compared with the automated count. Reviewer 2 appeared to be most consistently nearest the automated count.
4.3.3 Inter-operator variability in the nucleated red blood cell count

The mean nucleated red blood cell counts for all three reviewers were very different from the automated mean. On examination of the individual counts the automated analyser only recorded the presence of nucleated red blood cells in four cases. In contrast, nucleated red blood cells were recorded by all reviewers for 7 slides (slides 005D, 007D, 010D, 038D, 039D, 046D, 053D), and by at least one reviewer for 3 further slides (slides 022D, 049D, 079D). In two cases where nucleated red blood cells were recorded on the automated count these were low at 1 cell/100 white blood cells. These cases corresponded with slides 022D, 079D. For slide 022D manual counts of 0, 2 and 3 cells/100 white blood cells were recorded, whilst for slide 079D manual counts of 0, 0 and 2 cells/100 white blood cells were recorded. The corresponding mean nucleated red blood cell counts of 1.7 and 0.7 cells/100 white blood cells are therefore close to the machine count. In the other two cases where an automated nucleated red blood cell count was recorded (corresponding to slides 038D, 039D) the automated count appears much lower than the manual count. For slide 038D the mean manual count was 172 cells/100 white blood cells compared with an automated result of 38 cells/100 white blood cells. For slide 039D the mean manual count was 31 cells/100 white blood cells compared with an automated count of 10 cells/100 white blood cells. This is shown graphically in Figure 4-4.

Figure 4-4. Comparison of nucleated and automated nucleated red blood cell counts.
4.3.4 Inter-operator variability in reporting blasts

Blasts were not recorded in any of the automated reports. In contrast, blasts were reported on the manual count for 11 slides (slides 005D, 007D, 010D, 022D, 030D, 038D, 039D, 046D, 049D, 053D, 079D). Moreover, all reviewers agreed on the presence of blasts for nine of these, with only one reviewer not recording blasts for two of the slides (slides 007D, 038D).

4.3.5 Inter-operator variability in reporting morphological features

These were analysed by considering specific cell types. Unlike the white blood cell differential count where there is an automated count with which to compare the manual counts there was no external reference for these features.

1. Neutrophils – assessment of the degree of the following features was specifically requested: hypo or agranular neutrophils, hypersegmentation, Pelgeroid nuclei and vacuolation. Reviewer 1 appeared less likely to report these features compared with the reviewers 2 and 3 (slides 007D, 010D, 030D, 046D, 049D). There were no slides where everyone agreed on the grading of a feature. If grading was condensed so that occasional, some, moderate and plentiful were grouped together to indicate the presence of a feature, and if presence or absence only was compared then there was full agreement for just one slide (039D). Even with this, there was disagreement about the presence or absence of at least one of the neutrophil features in all the remaining slides.

2. Monocytes – assessment of the degree of the following features was specifically requested: vacuolation, stellate nucleus, an elongated nucleus, azurophilic granules. There were three slides with complete agreement between the three reviewers (005D, 039D, 053D). If just presence or absence was considered then there was full agreement in a further two slides (022D, 079D). The remaining discrepancies were between occasional/none, some/none and moderate/none. In three cases the discrepancy related to just one feature (030D, 038D, 046D).

3. Blasts – two questions were asked relating to blasts: can a type be ascribed; and, is there cytoplasmic blebbing. There was agreement between the three reviewers on the presence of cytoplasmic blebbing for four slides (030D, 039D, 046D, 079D). Reviewer 1 was alone in reporting cytoplasmic blebbing for a further five films (007D, 010D, 022D, 038D, 049D), although curiously did not for one film where both other reviewers reported blebbing (053D). There was disagreement in blast type in one film between myeloblasts and monoblasts (005D).
4. Red blood cells – grading of the following features was requested: macrocytosis, polychromasia, poikilocytosis, basophilic stippling and target cells. There was only one slide on which all three reviewers agreed (007D). If the grading was condensed to consider just presence or absence then there was agreement in a further four cases (005D, 010D, 022D, 079D). The most common discrepancy was in whether basophilic stippling was or was not seen (038D, 039D, 049D). The discrepancies tended to be between similar grades, for example some/occasional and were never between moderate/none.

5. Platelets – the reviewers were asked whether giant platelets and megakaryocyte fragments were present, whether the platelet number appeared consistent with the automated count, and whether there was a change in platelet colour. There was agreement about the presence of giant platelets between all three reviewers in almost all slides, with a discrepancy in just three slides (049D, 053D, 095D) - although in one of these the recording of no giant platelets was qualified by the comment ‘slightly bigger’ (049D). The reporting of megakaryocyte fragments was very variable between reporters. Reviewer 2 was more likely to report megakaryocyte fragments, being the only person to report them in three slides (005D, 007D, 039D). Reviewer 3 was the least likely to report megakaryocyte fragments only reporting them in one slide (046D), and saying ‘difficult to distinguish’ when both other reviewers saw them (079D).

4.3.6 Intra-operator variability

A further six slides were reviewed twice by the same reviewer, reviewer 2, to assess intra-operator variability (slides 058D, 061D, 073D, 076D, 086D, 088D). The reviews were on separate dates and with no knowledge of what had been written down before.

In contrast to the inter-operator analysis there were very few discrepancies. The following were noted in assessment of the morphological features:

1. The only discrepancies for reporting neutrophil features were between some/none.
2. There was agreement for most other white blood cell features. There was a discrepancy between some/occasional for monocytic features and just one some/none discrepancy for the presence of cytoplasmic blebbing.
3. There was only one disagreement as to whether a red blood cell feature was present or absent, again relating to basophilic stippling. Otherwise there was full agreement for red blood cell features if some/occasional were grouped and if some/moderate were grouped.
4. There was full agreement for platelets if were grouped. If grading was condensed so that some/occasional, some/moderate, and moderate/plentiful were grouped together then there was full agreement for platelet features.

Overall, there was a higher degree of intra-observer consistency compared with inter-observer consistency. However, it was clear that grading still posed a problem. Where this varied between occasional, some or moderate it was at least simply an assessment of degree that varied. However, for some of the neutrophil features, and for one occurrence of basophilic stippling the variation was still between some and none.

4.3.7 Errors inherent to the process

There were both random and systematic errors present relating to the way that the review had been set up. Random errors occur when a measured value differs from the true value because of chance. These occurred here particularly because a limited number of white blood cells are reviewed manually – the automated differential white blood cell count is based on at least 100 fold more white blood cells than the manual count. The particular 100 or 200 white blood cells included in the count may vary depending on the part of the film reviewed, the way the film is spread or chance.

Systematic errors, or bias, occur when measured values differ from the truth in a systematic manner. In fact, here ‘truth’ was not well defined. This is seen in three key areas:

1. There was no stated plan for review; instead it was assumed that each would count a similar number of white blood cells, however, reviewing the completed assessments it became apparent that the number reviewed varied from 50-200, compounding the random error above.

2. There were no stated cell definitions; again it was supposed that all would identify a white blood cell or a morphological abnormality in a similar manner. Although there might be thought to be, and appeared to be, some ground for this assumption when considering the identification of a white blood cell some of the other changes were more subtle and systematic differences became evident on the analysis.

3. Descriptive terms were imprecise - it was not clear whether, for example, ‘some’ monocyte vacuolation meant that some of the monocytes were vacuolated, or whether it meant that some of each monocyte was vacuolated.
4.3.8 Errors specific to individual reviewers

It had been assumed that each reviewer would complete the morphology assessment in a comparable fashion. The systematic errors evident in the inter-operator assessment, which were compounded by the lack of clarity of instruction and definition as described above, illustrate how unfounded this assumption was.

A further area in which this was apparent was in the grading system. When grading the degree of a feature judgement tended to be retrospective and based on an impression - different versions of the proforma had used a percentage or a word to try to grade a feature, for example reporting neutrophil hypogranulation as moderate. However, this classification tended to be a retrospective judgement based on the overall impression, and so was very subjective.

There was also an explicit tendency for at least one reviewer to compare what was seen with an internal idea of what was normal based on experience – the reviews had been completed by senior paediatric haematologists with considerable experience in looking at neonatal blood films. From some of the comments it became clear that at times the appearances were being internally calibrated against experience so that the degree of a feature was being recorded in relation to what was expected and not as an absolute feature.

4.3.9 Errors relating to the automated count

The analysis of the white blood differential results also highlighted systematic errors inherent in the automated count. These particularly related to identification of neutrophil precursors, of blasts and of nucleated red blood cells.

The automated count did not differentiate well between mature neutrophils and neutrophil precursors compared with the manual count. Most samples were processed on the Advia 2120, which aims to flag the presence of immature granulocytes, but not to quantify them (Harris et al, 2005). It uses two methods to produce the white blood cell differential. The primary method is based on cytochemistry and measures light generated from a reaction involving intracellular myeloperoxidase to differentiate between cell types. The second method uses a reagent to lyse the cells before measuring high angle light scatter (relating to the nuclear contents) and low angle light scatter (relating to cell size) which is depicted graphically.

Although the lack of quantification of neutrophil precursors is therefore not surprising for the Advia 2120, one of the advantages of the Sysmex XE-2100 is supposed to be its ability to
quantify immature myeloid and lymphoid cells, as well as identifying the five main white blood cell sub-populations (neutrophil, basophil, eosinophil, monocyte and lymphocyte) (Herklotz and Huber, 2001). The Sysmex XE-2100 also uses two methods to generate a differential white blood cell count. Light from a semi-conducting laser is used to examine each cell, and then a cell type is assigned based on the combination of two out of three of forward scatter (which relates to cell volume), side scatter (which relates to the cell contents), and side fluorescence (which relates to the amount of RNA and DNA within the cell) (Herklotz and Huber, 2001). If there are abnormalities flagged in one channel then this should be corrected by the other channel as the immature granulocyte is specifically calculated and is then subtracted from the neutrophil total.

The presence of blasts was not reliably picked up by the automated analyser. There are few reports of the performance of either the Advia 2120 or the Sysmex XE-2100 in detecting blasts. A recent study of the Advia 2120 found a sensitivity of 100%, but with relatively low specificity of 49% (Shelat, Canfield and Shibutani, 2010). This would suggest that false positives are more likely and does not explain the findings here. The performance of the Sysmex XE-2100 specifically in identifying blasts was evaluated using samples with a manual blast count of >10% (Jung et al, 2010). The Sysmex recognised that there was a white cell abnormality in 72% cases, resulting in it reporting an invalid white blood cell differential count. Interestingly, in the samples for which it did produce a differential count it tended to assign lymphoblasts as lymphocytes and myeloblasts as monocytes. This report compares with an earlier study which compared the Sysmex XE-2100 with three other analysers (Kang et al, 2008). The Sysmex performed best with a sensitivity for identifying blasts of 90.9%.

The automated count did not always report nucleated red blood cells compared with the manual count. As this interim analysis occurred in the early stage of the study most samples were processed on an Advia 2120 with only three samples processed using a Sysmex XE-2100. A previous study of the performance of the Advia 2120 in reporting nucleated red blood cells compared with a manual count as a gold standard reported a sensitivity and specificity of 77.3% and 74.6% respectively (Kratz et al, 2006). Importantly, most of the false negative samples were at nucleated red blood cell counts ≤ 10/100 white blood cells. This would account for most of the false negatives seen here (slides 007D, 010D, 049D, 053D). Nucleated red blood cells were reported on all three of the samples processed on the Sysmex XE-2100. This uses a separate channel to count the nucleated red blood cells with a specific reagent to lyse red blood cells and then to denucleate, shrink and stain the nuclei of nucleated red blood cells allowing enumeration. Importantly, this also stains the nuclei and
intracytoplasmic organelles of white blood cells, but does not alter their size or shape allowing them to be distinguished from the red blood cells. The nucleated red blood cells calculated in this channel are subtracted from the lymphocyte total generated initially from flow cytometry as it will have included them.

The high performance of the Sysmex XE-2100 in detecting low levels of nucleated red blood cells has been demonstrated elsewhere (Ruzicka et al., 2001; Briggs et al., 2000; de Keijzer and van der Meer, 2002) and is consistent with these results. Interestingly, it has also been reported that the Sysmex XE-2100 tends to record a lower nucleated red blood cell count in samples with a high manual count - >15 cells/100 white blood cells and especially where the manual count is >200 cells/100 white blood cell count (Ruzicka et al., 2001) which accords with the experience here. The remaining incongruity, for slide 046D, where all three reviewers reported nucleated red blood cells but there does not appear to be an automated result can be explained by the absence of a machine count as the sample was clotted.

Overall, the Sysmex XE-2100 appears better at picking up low level presence of nucleated red blood cells compared with the Advia 2120. However, it is not accurate at higher counts and a manual count is required for enumeration if the nucleated red blood cell count is >15 cells/100 white blood cells.

4.3.10 Summary

The initial approach taken with the morphology review was to design a proforma based on those used for blood cell morphology review in the UK Medical Research Council trials. However, it was rapidly apparent that there were frequent discrepancies between the results recorded by the three reviewers with unacceptable levels of intra- and inter-operator variability. The factors contributing to this were examined in order to inform the development of a new strategy for morphology review.
4.4 Development of a new morphology reporting tool

4.4.1 Rationale for revision

The previous approach to morphology review had been based on the proformas used in major trials, adapted to be relevant to this setting. This had so clearly failed to be useful that a wholly new approach was needed. First, the requirements of the morphological review were defined. This was primarily to enable objective analysis of the blood cell morphology of DS neonates within the study context. However, it was also hoped that this new tool would be able to be used outside of the study context by a qualified haematologist or laboratory scientists with morphological expertise, to give similar results.

4.4.2 Key elements of the new reporting tool

The new tool for morphology review assumed as little as possible and had a standard operating procedure for analysis. The key elements of this were as follows:

1. The conditions that needed to be met for analysis to proceed were explicitly stated.
2. Given the problems described in the automated count with regard to the differential white blood cell count, identifying blasts and in enumerating nucleated red blood cells these were all to form part of the review.
3. The number of white blood cells counted in the differential was stipulated as 100 white blood cells.
4. The nucleated red blood cell count was to be recorded per 100 white blood cells, and not as part of a total nucleated cell count.
5. There were clear definitions of each abnormal feature considered.
6. Importantly, in the new approach it was decided that each individual white blood cell would be reviewed as it was counted for the presence or absence of the particular features under consideration for that cell type. The end number, for a specific feature, would then accurately refer to the percentage of cells with that feature for a given cell type.
7. The number of fields to be reviewed for red blood cell and platelet features was stipulated as 20 fields at high power (x50).

The format of the revised morphology reporting tool is seen in Figure 4-5. The instructions for the new morphology review procedure are provided in Appendix 9.
Figure 4-5. Revised morphology review tool.

**Morphology review proforma**

*Slide identification and processing*
1. Case ID: 
   - Review: date sample taken: 
   - date processed: 
   - age of child when taken: <14d/ <6m/ <1y/ <2y/ <3y/ <4y/ <5y

*Slide suitability*
2. Macroscopic:
   - Is the spreading satisfactory? yes/no
   - Is the staining satisfactory? yes/no
3. Microscopic:
   - Is the spreading satisfactory? yes/ no
   - Is there evidence of storage artefact? yes/ no

4. Overall, is the slide suitable for review? yes/ no / partial review

*Morphology analysis*

*Nucleated cell count*
5. Can a differential count be determined? yes/ no

100 white blood cells to be counted and assigned. As each mature cell is viewed please tick overleaf to indicate if specific features are present.

<table>
<thead>
<tr>
<th>neutrophils</th>
<th>basophils</th>
<th>eosinophils</th>
<th>monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>lymphocytes</td>
<td>band forms</td>
<td>myelocyte</td>
<td>metamyelocyte</td>
</tr>
<tr>
<td>promyelocyte</td>
<td>lymphoblast</td>
<td>myeloblasts</td>
<td>blast - other</td>
</tr>
</tbody>
</table>

**Nucleated red cells/100 wbc**

<table>
<thead>
<tr>
<th>White blood cells</th>
<th>number</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypergranular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypogranular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>agranular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypersegmentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelger forms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vacuolation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>free text</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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<th>number</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>vacuolation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>free text</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophils</td>
<td>vacuolation</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
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</tr>
<tr>
<td></td>
<td>dysplasia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>abnormal granulation</td>
<td></td>
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<tr>
<td></td>
<td>free text</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>vacuolation</td>
<td></td>
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<tr>
<td></td>
<td>dysplasia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>abnormal granulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pelger type nuclei</td>
<td></td>
</tr>
<tr>
<td></td>
<td>free text</td>
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</tr>
<tr>
<td>Monocytes</td>
<td>vacuolation</td>
<td></td>
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<tr>
<td></td>
<td>stellate forms</td>
<td></td>
</tr>
<tr>
<td></td>
<td>elongated lobes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>azurophilic granules</td>
<td></td>
</tr>
<tr>
<td></td>
<td>free text</td>
<td></td>
</tr>
</tbody>
</table>

Overall, was there granulocytic nuclear/cytoplasmic asynchrony? Yes/ no

Blasts
1) Is there cytoplasmic blebbing? Yes/ no
2) Circle blast type: lymphoblasts, myeloblasts, eo-myeloblasts, cannot ascribe
3) Free text

Red blood cells

<table>
<thead>
<tr>
<th></th>
<th>0 none</th>
<th>1 &lt;5%</th>
<th>2 5-24%</th>
<th>3 25-49%</th>
<th>4 ≥ 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrocyes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polychromasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophilic stippling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Are there other abnormal RBC yes/no
If yes then circle echinocytes, spherocytes, Howell Jolly, other
Free text

Platelets
4) Are there giant forms? yes/no
5) Are there megakaryocyte fragments? yes/no
6) Is the automated platelet count reliable yes/ no
7) Is the platelet colour abnormal yes/ no
8) If yes the platelets very pale/pale/other abnormality
9) Free text

Pictures taken? Yes/ no
Review done by _______________________
Date _______________________

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4.5 Evaluation of the revised morphology reporting tool

The revised morphology reporting tool was used to report twenty four blood films from an unselected group of neonates having full blood counts and films made at Leeds General Infirmary. The films had been made and stained according to a standard operatory procedure using a Leishman stain (Lewis et al, 2006). Each slide was reported manually three times: reviews 1 and 2 were by the same reviewer at separate times; review 3 was by a different reviewer (this was reviewer 2, whose consistency had been demonstrated in the analysis above). Comparison of reviews 1 and 2 enabled assessment of intra-operator variability, while comparison of these with review 3 enabled assessment of inter-operator variability.

4.5.1 Comparison of the manual and automated differential white blood count

Initially, the mean value for each white blood cell type, and for blasts, was calculated for each of the reviewers and these were compared with the automated result. This is shown in Figure 4-6.

![Figure 4-6. Comparison of the manual and automated counts using the new morphology reporting tool.](image-url)
In contrast to the results obtained with the initial proforma these were very similar and so a more detailed slide by slide comparison was made. First, the differential count for each white blood cell type recorded at each individual slide review was compared with the automated differential count for that cell type associated with each slide. The automated count was taken as the baseline. The deviation of each of the white blood cell differential from the automated baseline is shown, for each cell type and for each slide, for reviews 1, 2 and 3 in Figure 4-7.

All of the results were within 20% of the baseline – the pre-set level of agreement considered to be satisfactory. In fact, only one result approached this. Almost all the results for the neutrophils, lymphocytes and monocytes were within 10% of the baseline, while all the results for eosinophils, basophils and blasts were within 5% of the baseline.

Importantly, the results were variably greater than and less than the baseline – a pattern consistent with random rather than systematic error. All three reviews appeared comparable in their similarity to the automated count. There was not one series, or one reviewer, which looked obviously different.
Figure 4-7. Slide by slide comparison of the % difference in each of the manual white blood cell differential counts compared with the automated count which was taken as the baseline.

Each bar represents the deviation for one slide. Where there is no bar this indicates that there was no difference between the manual and the automated count.

Review 1 =  Review 2 =  Review 3 =  

A. Neutrophils
B. Lymphocytes
C. Monocytes
D. Eosinophils
E. Basophils
F. Blasts
Although there is close agreement between the three manual reviews and the automated count for the total neutrophil and neutrophil precursor count, when this is broken down into neutrophils, myelocytes, metamyelocytes, promyelocytes and blasts differences emerge. The data suggests that the automated count tends to consistently overestimate the mature neutrophils and to underestimate the neutrophil precursors. This effect becomes more pronounced as the cell type being counted becomes more immature – especially given that the numbers become much smaller as the type considered is more immature. Metamyelocytes, promyelocytes and blast counts were all lower for the automated compared with the manual counts. For example, blasts were present in three of the slides. These were consistently identified by all three reviewers, but were identified in the automated differential count in only one case, slide 018M. This was the slide which had the highest manual blast count, with a mean blast count of 4%. When the blast percentage was lower than this the machine did not identify the presence of blasts.

This is illustrated in Figure 4-8. All the results (the automated result and the three manual results) appear very similar when the total mature and immature neutrophil count is considered. However, when this is broken down it can be seen that the mature neutrophil count is higher for the automated result and the precursors are lower when counted by the automated analyser.

![Figure 4-8. Comparison of the mean mature and immature neutrophil manual counts for each review with the automated counts which is taken as the baseline. Review 1 =  Review 2 =  Review 3 = ]
4.5.2 Comparison of the manual differential white blood cell counts

A slide by slide comparison of the individual manual reviews was then made. Review 1 was taken as a baseline, and review 2 was compared with it to assess intra-operator variability. Review 3 was compared with review 1 in order to assess inter-operator variability. This is shown graphically in Figure 4-9.

All of the results were within 20% of the baseline – the pre-set level of agreement considered to be satisfactory. Almost all the results for the neutrophils, lymphocytes and monocytes were within 10% of the baseline, while all the results for eosinophils, basophils and blasts were within 5% of the baseline.

Again, the results were variably greater than and less than the baseline – a pattern consistent with random rather than systematic error. This indicated that there was good intra-operator and inter-operator reliability.
Figure 4-9. Slide by slide comparison of the % difference in each of the manual white blood cell differential counts for reviews 2 and 3 compared with review 1 which was taken as the baseline.

Each bar represents the deviation for one slide. Where there is no bar this indicates that there was no difference between the manual counts.

Intra-operator comparison = ■ Inter-operator comparison = ▼
4.5.3 Assessment of morphological features in the neutrophils

In order to assess the intra-operator variability the results for the percentage of cells noted to have the feature under consideration was calculated and then these results were compared slide by slide. Review 1 was taken as a baseline, and review 2 was compared with it to indicate intra-operator variability. Review 3 was compared with review 1 in order to assess inter-operator variability. The comparisons are shown graphically in Figure 4-10.

Each neutrophil was assessed to look for any of six possible features: hypersegmentation, hypogranularity, agranularity, hypergranularity, Pseudo-Pelger Hűet anomaly and vacuolation. There were very few slides which had any agranular or hypersegmented neutrophils, and so there were only a handful of slides for which these numbers could be compared.

Most of the results were within 10% of the baseline, although three results were just outside 20% of the baseline.

This indicated that there was reasonable intra-operator and inter-operator reliability.
Figure 4-10. Intra and inter-operator variability in the assessment of specific neutrophil features.

Where there is no bar this indicates that there was no difference between the manual counts.

Intra-operator comparison = ■ Inter-operator comparison = □
4.5.4 Assessment of morphological features in lymphocytes, monocytes, eosinophils and basophils

The total numbers of lymphocytes, monocytes, eosinophils and basophils seen are small and the frequency of most of the features seen was low. This means that small numerical differences in the number of cells reported to have a certain feature can make large percentage changes – for example, if 4 eosinophils are seen in a 200 white blood cell differential and none are noted to be vacuolated then the percentage vacuolation is 0%, but if just 2 are noted to be vacuolated this rises dramatically to 50%. This is illustrated in which shows the variation in the percentage of monocytes with vacuolation against the average number of monocytes for that slide. As the number of monocytes examined increases the variation decreases. The graph also shows that the results are less consistent than those for the differential white blood cell count, with many of the results from reviews 2 and 3 being >20% from the baseline.

Figure 4-11. Variation in the monocyte count from baseline against the total monocyte count.
4.5.5 Assessment of morphological features in the red blood cells

Red blood cells were assessed so that a grade was assigned for macrocytosis, polychromasia, basophilic stippling and target cells—the grade indicated the fraction of the total red blood cell population estimated to display this feature. The presence or absence of spherocytes and Howell-Jolly bodies was noted, but not graded. The analysis of nucleated red blood cells is considered separately below. Review 1 was taken again as a baseline, and review 2 was compared with it to indicate intra-operator variability and review 3 compared with it to indicate inter-operator variability. The results are shown in Table 4-1.

When intra-operator variability was considered, there was complete agreement in the grading of macrocytosis in 21/24 (87.5%) cases; in the grading of polychromasia in 22/24 (91.7%) cases; in the grading of basophilic stippling in 22/24 (91.7%) cases; in the grading of target cells in 21/24 (87.5%) cases; in the recognition of spherocytes in 23/24 (95.8%) cases; and in the recognition of Howell-Jolly bodies in 22/24 (91.7%) cases. When the degree of discrepancy was considered it was apparent that this was only ever of one grade—for example one review grading macrocytosis as grade 2 whilst the other review gave a grade of 3.

When inter-operator variability was considered, there was complete agreement in the grading of macrocytosis in only 10/24 (41.7%) cases; in the grading of polychromasia in 12/24 (50%) cases; in the grading of basophilic stippling in 20/24 (83.3%) cases; in the grading of target cells in 14/24 (66.7%) cases; in the recognition of spherocytes in 15/24 (62.5%) cases; and in the recognition of Howell-Jolly bodies in 18/24 (75%) cases. More detailed analysis showed that there appeared to be systematic errors in the inter-operator comparison with review 3 being less likely to report the presence of any of the red blood cell features compared with reviews 1 or 2. When the degree of discrepancy was considered it was apparent that although this was almost always only a difference of 1 grade, there were three instances where the grade differed by 2 points, and one case where the grade differed by 3 points.
Table 4-1. Agreement in the identification and grading of red blood cell and platelet features.

<table>
<thead>
<tr>
<th>Feature being considered</th>
<th>Intra-operator agreement</th>
<th>Inter-operator agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Macrocytosis</td>
<td>21/24</td>
<td>87.5</td>
</tr>
<tr>
<td>Polychromasia</td>
<td>22/24</td>
<td>91.7</td>
</tr>
<tr>
<td>Basophilic stippling</td>
<td>22/24</td>
<td>91.7</td>
</tr>
<tr>
<td>Target cells</td>
<td>21/24</td>
<td>87.5</td>
</tr>
<tr>
<td>Spherocytes</td>
<td>23/24</td>
<td>95.8</td>
</tr>
<tr>
<td>Howell-Jolly bodies</td>
<td>22/24</td>
<td>91.7</td>
</tr>
<tr>
<td>Giant platelets</td>
<td>22/24</td>
<td>91.7</td>
</tr>
<tr>
<td>Megakaryocyte fragments</td>
<td>24/24</td>
<td>100</td>
</tr>
<tr>
<td>Platelet colour</td>
<td>24/24</td>
<td>100</td>
</tr>
</tbody>
</table>

Macrocytosis is also assessed by the automated machine which calculates the mean cell volume. When the grade assigned at each of the three reviews was plotted against the mean cell volume ascribed by the automated machine it was apparent that there was a linear relationship between the grade and the mean cell volume. This is shown in Figure 4-12.
Figure 4-12. Relationship between the manual grade for red blood cell macrocytosis and the mean cell volume calculated by the automated analyser.

4.5.6 Assessment of the nucleated red blood cells

The nucleated red blood cell count for each of the reviewers was compared with the automated result, which was taken as the baseline. This is shown in Figure 4-13.

![Figure 4-13](image)

*Figure 4-13. Slide by slide comparison of the % difference in the manual nucleated red blood cell count compared with the automated count, which was taken as the baseline. Review 1 = Review 2 = Review 3 =*

There was good correlation between the nucleated red blood cell count obtained for all three manual reviews, indicating good intra and inter-operator variability. For most of the slides there was also a good correlation of all three manual counts compared with the automated count – with most of these falling within 10% of the automated count. These were variably 10% more or less than the automated count indicating that this was random error. However, for manual reviews of 5 slides the manual count was >10% different from the automated count, and when this was so the manual count was always lower than the automated count. In some cases (review 1 and 2 of slide 3; review 2 of slide 18) the manual count differed by >40% from the automated count.

This contrasts with the experience of the Sysmex XE-2100 automated count in the initial reviews where the results indicated that the manual nucleated red blood cell count was underestimated by the Sysmex XE-2100 if there was a high nucleated red blood cell count – a finding which has been reported elsewhere too (Ruzicka et al, 2001). However, all of the blood samples associated with the slides examined in this validation exercise were processed on the Advia, and not on the Sysmex XE-2100.
4.5.7 Assessment of platelet features

Platelets were assessed with regard to size and colour. Review 1 was taken again as a baseline, and review 2 was compared with it to indicate intra-operator variability and review 3 compared with it to indicate inter-operator variability. The results are shown in Table 4-1.

When intra-operator variability was considered, there was agreement in the presence of giant platelets in 20/22 (90.9%) cases; in the presence of megakaryocyte fragments in all cases; and in the colour in all cases. When the only two discrepancies were considered these indicated a random error.

When inter-operator variability was considered, there was agreement in the presence of giant platelets in 16/22 (72.8%) cases; in the presence of megakaryocyte fragments in 20/22 (90.9%) cases; and in the assessment of colour in 20/22 (90.9%) cases. Again there was no pattern to the discrepancies consistent with random error.
4.6 Final refinements and conclusions

The evaluation exercise demonstrated that it is possible to standardise blood morphology review so that the results are reproducible. This is essential if results are to be compared in any meaningful way.

4.6.1 The value of the manual compared with the automated count

Morphological analysis is labour intensive and time consuming – each review took ~15 minutes to complete. This is only worthwhile if the manual result is superior to the automated result and/or it yields extra, helpful, information.

The overall differential white blood cell count was comparable whether assessed manually or using the automated analyser. However, the automated count did not offer a satisfactory breakdown of neutrophil precursors, and the manual count was superior in identifying these. This effect became more marked with increasing immaturity and the manual review was superior in recognising the presence of blasts, when they were present at low levels.

The manual review was also superior in identifying and counting nucleated red blood cells. When these were present at low levels they were not reliably recognised by the automated analyser. When they were present at high levels there was a tendency for the Advia 2120 to over count these, whilst the Sysmex XE-2120 has been shown to undercount these. The other parameter that could be compared between the manual and the automated result was the degree of macrocytosis. There was no added value from commenting on the degree of red blood cell macrocytosis as it appears morphologically as this correlated directly with the automated mean red blood cell volume.

The automated machine is not sufficiently sophisticated to be able to comment on the presence or absence of morphological features within the blood cells. The main questions with regard to these are therefore:

1. Can these be reliably assessed?
2. If so, do they add important information?

4.6.2 Intra-operator variability

The intra-operator variability was acceptable for all aspects of the review, with the exception of the identification of morphological abnormalities within the lymphocytes, monocytes, eosinophils and basophils. These became more reliable at higher counts – or when the monocyte population was a greater proportion of the total white blood cell differential.
However, in ~one fifth of cases the variation was >20% baseline. This was deemed unacceptable.

### 4.6.3 Inter-operator variability

The inter-operator variability was acceptable for the differential white blood cell count, and for the identification of morphological abnormalities in the neutrophils. As above, the identification of morphological abnormalities within the lymphocytes, monocytes, eosinophils and basophils was not reliable. There was a much greater inter-operator variability in the assessment of red blood cell morphology. Importantly, this also showed a consistent trend with review 3 being less likely to report an abnormality and being more likely to give a lower grade when considering an abnormality. This was therefore a systematic error. Reviewer 3 was similarly less likely to report giant platelets although there was only a low level of inter-operator variability when considering platelet colour.

### 4.6.4 Final refinements

In the light of the above the following refinements were made:

1. Morphological features in the lymphocytes, monocytes, eosinophils and basophils were not considered;
2. The degree of macrocytosis was not graded as the machine mean cell volume was already being recorded;
3. All reviews would be conducted by the same operator.

### 4.7 Summary

The interim analysis threw up an unexpected problem, namely the unreliability of the morphology results obtained using the initial proforma. Although morphology is an explicitly subjective measure the extent of this came as a surprise as forms similar to the initial proforma are used in other studies and trials.

The morphology review tool that was developed and tested here on a group of unselected neonatal slides demonstrated that it is possible to record the results of morphological analysis in a way that is sufficiently reliable that there can be meaningful analysis. Moreover, the evaluation exercise proved that the manual differential count is important as it yields information which the automated count either cannot, or cannot reliably, contribute. In particular, it provides important information about neutrophil precursors, blasts and the nucleated red blood cells.
Chapter 5 Results: study participation and case demographics

5.1 Introduction

In the first part of this chapter the results of the pilot phase are presented, before concentrating on the CDSS as a whole.

5.2 Pilot phase results

5.2.1 Hospital participation within the Yorkshire Neonatal Network

The study opened in two hospitals on 1st May 2006. The final research and development approval for a further five hospitals within the YNN was received during May 2006, for a further four hospitals in June 2006 and for the final hospital in early July 2011.

5.2.2 Hospital participation outside the Yorkshire Neonatal Network

In addition, as contact with hospitals in the wider target area continued in parallel with the pilot study the study was also open in a further 29 hospitals by the end of the pilot study. A presentation about the study had been made in 8 further hospitals, 7 of which had agreed to proceed, identified a local lead and for which I had submitted research and development approval. The eighth hospital were keen to participate, but had not yet nominated a local lead. Contact had been made successfully, and a date set to present the study had been agreed with 7 more hospitals. A date to present the study had not been made in the final 5 hospitals within the target area, although contact had been established with each of these.

5.2.3 Recruitment within the Yorkshire Neonatal Network

Sixteen children were recruited within the YNN during the 8 months of the pilot study. Details of the recruitment by hospital and data on the live birth rates are presented in Table 5-1. These indicate an estimated number of 25 live births would have been expected within these hospitals during this period, giving an estimated ascertainment of 64% within the YNN.

5.2.4 Recruitment outside the Yorkshire Neonatal Network

During the pilot phase 17 children were recruited from hospitals outside the Yorkshire Neonatal Network. Details of the recruitment by hospital and data on the live birth rates for hospitals joining the study during the pilot period, but outside the Yorkshire Neonatal Network are presented in Table 5-2. These indicate an estimated number of 23 live births would have been expected within these hospitals during this period, giving an estimated ascertainment of 74% for this group of hospitals, and an overall estimated ascertainment of 69% (33/48).
Table 5-1. Recruitment for hospitals in the Yorkshire Neonatal Network during the pilot phase.

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Date study opened</th>
<th>Duration opened by review point (months)</th>
<th>Registered live births pa&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Estimated number of DS live births in pilot phase</th>
<th>Cases recruited to the CDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1</td>
<td>26/05/2006</td>
<td>7</td>
<td>2394</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>Y2</td>
<td>26/06/2006</td>
<td>6</td>
<td>5746</td>
<td>3.2</td>
<td>2</td>
</tr>
<tr>
<td>Y3a</td>
<td>03/05/2006</td>
<td>7</td>
<td>5441&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5</td>
<td>1</td>
</tr>
<tr>
<td>Y3b</td>
<td>03/05/2006</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Y4a</td>
<td>11/05/2006</td>
<td>7</td>
<td>6189&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.0</td>
<td>0</td>
</tr>
<tr>
<td>Y4b</td>
<td>11/05/2006</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Y5</td>
<td>11/07/2006</td>
<td>5</td>
<td>1713</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>Y6</td>
<td>14/06/2006</td>
<td>6</td>
<td>5384</td>
<td>3.0</td>
<td>2</td>
</tr>
<tr>
<td>Y7a</td>
<td>01/05/2006</td>
<td>8</td>
<td>8786&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.4</td>
<td>1</td>
</tr>
<tr>
<td>Y7b</td>
<td>01/05/2006</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Y8</td>
<td>26/06/2006</td>
<td>6</td>
<td>1615</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>Y9</td>
<td>26/06/2006</td>
<td>6</td>
<td>3242</td>
<td>1.8</td>
<td>1</td>
</tr>
</tbody>
</table>

| Total    |                    |                                        |                                  | 25.1                                            | 16                       |

<sup>a</sup> Data taken from www.hesonline.nhs.uk using 2006-2007 statistics with the exception of Hospital Y8 as the statistics were inadvertently incorrectly recorded by the Trust that year. Instead, data for Hospital Y8 is taken from the year 2008-2009.

<sup>b</sup> Hospitals Y3a and Y3b belong to the same Trust and these are the combined number of live births.

<sup>c</sup> Hospitals Y4a and Y4b belong to the same Trust and these are the combined number of live births.

<sup>d</sup> Hospitals Y7a and Y7b belong to the same Trust and these are the combined number of live births.
Table 5-2. Recruitment for hospitals outside the Yorkshire Neonatal Network during the pilot phase.

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Date study opened</th>
<th>Duration opened by review point (months)</th>
<th>Registered live births pa(^a)</th>
<th>Estimated number of DS live births in pilot phase</th>
<th>Cases recruited to the CDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM3</td>
<td>21/06/2006</td>
<td>6</td>
<td>2753</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>CM4</td>
<td>26/10/2006</td>
<td>2</td>
<td>7994</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>CM7</td>
<td>16/11/2006</td>
<td>1</td>
<td>3051</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>CM8</td>
<td>19/12/2006</td>
<td>0</td>
<td>3002</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M2b</td>
<td>01/12/2006</td>
<td>1</td>
<td>5183(^b)</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>M2c</td>
<td>10/10/2006</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>M4</td>
<td>18/09/2006</td>
<td>3</td>
<td>4996</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td>M5</td>
<td>21/11/2006</td>
<td>1</td>
<td>3514</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>M7</td>
<td>22/12/2006</td>
<td>0</td>
<td>4097</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M8</td>
<td>07/08/2006</td>
<td>4</td>
<td>1377</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>NT1</td>
<td>22/06/2006</td>
<td>6</td>
<td>2571</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td>NT3</td>
<td>16/11/2006</td>
<td>1</td>
<td>2858</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>NT4a</td>
<td>15/06/2006</td>
<td>6</td>
<td>4398(^c)</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>NT4b</td>
<td>15/06/2006</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>NT6</td>
<td>25/08/2006</td>
<td>4</td>
<td>6530</td>
<td>2.4</td>
<td>1</td>
</tr>
<tr>
<td>NT7</td>
<td>24/08/2006</td>
<td>4</td>
<td>2685</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>N1b</td>
<td>09/11/2006</td>
<td>1</td>
<td>5729(^d)</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>N1c</td>
<td>09/11/2006</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>N2a</td>
<td>03/10/2006</td>
<td>2</td>
<td>2990(^e)</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>N2b</td>
<td>03/10/2006</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>N6</td>
<td>14/11/2006</td>
<td>1</td>
<td>1764</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>N7</td>
<td>20/09/2006</td>
<td>3</td>
<td>5494</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>N8</td>
<td>02/06/2006</td>
<td>6</td>
<td>1488</td>
<td>0.8</td>
<td>2</td>
</tr>
<tr>
<td>N10a</td>
<td>14/11/2006</td>
<td>1</td>
<td>3727(^f)</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>N10b</td>
<td>14/09/2006</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>T2</td>
<td>27/10/2006</td>
<td>2</td>
<td>5304</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>T3</td>
<td>11/09/2006</td>
<td>3</td>
<td>2668</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>T4a</td>
<td>23/08/2006</td>
<td>4</td>
<td>10 105(^g)</td>
<td>3.7</td>
<td>2</td>
</tr>
<tr>
<td>T4b</td>
<td>23/08/2006</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>23.3</strong></td>
<td><strong>17</strong></td>
<td><strong>23.3</strong></td>
<td><strong>17</strong></td>
</tr>
</tbody>
</table>


\(b\). Hospitals M2b and M2c belong to the same Trust and these are the combined number of live births.
c. Hospitals NT4a and NT4b belong to the same Trust and these are the combined number of live births.
d. Hospitals N1b and N1c belong to the same Trust and these are the combined number of live births.
e. Hospitals N2a and N2b belong to the same Trust and these are the combined number of live births.
f. Hospitals N10a and N10b belong to the same Trust and these are the combined number of live births.
g. Hospitals T4a and T4b belong to the same Trust and these are the combined number of live births.

5.2.5 Protocol amendments

Change of hospital laboratory

The first test to be performed on each blood sample is full blood count processing, a test which is routinely performed in hospital haematology laboratories. Initially the study was set up so that blood samples were sent to the hospital haematology laboratory at SJUH for full blood count testing. This seemed appropriate as there would be consultant paediatric haematologists on site to review results immediately if there were concerns. Following processing the samples were collected and taken to the ECSG at the University of York for storage. Although, there is close collaboration between the ECSG and the Regional Centre for Paediatric Haematology at SJUH on a number of projects, they are 25 miles apart. Over the first few months transport problems with this arrangement, and so for logistical reasons a decision was made to change the location so that samples would be sent instead to the smaller hospital haematology laboratory at York Hospital, which is geographically close to the University. The newborn entry packs which are kept at participating hospitals were recalled and reissued once the prepaid addressed envelopes had been amended. This new set up worked extremely well. Communication between the hospital laboratory and the study centre was excellent, so that it became possible for samples to reach the study centre having had a full blood count test and slides made, within 48 hours of venepuncture. HPLC had already been completed on twenty samples by the time the system changed.

Inclusion of access to maternity records in the newborn consent

As the information from maternity notes pertains directly to the newborn sample, it was felt more appropriate that permission to review these maternity records was also sought when the family was initially invited to take part and so this was added to the newborn consent form.

More flexibility over timing of the move to the follow up stage

It was also decided to introduce more flexibility into the timing between the newborn and the follow up stage. In the original design there was a set interval of three months between the initial participation—which typically occurred when the family were in hospital with their
newborn baby – and the next contact from the study centre when families were invited to take part further. Before contact was made the study centre would check with the relevant health professional, usually the general practitioner, that there was nothing new in the families’ circumstances that would make this contact inappropriate. The protocol was amended so that after the initial participation, in addition to a letter thanking them for taking part, families were sent a prepaid addressed card which they could return when they would like to find out more about the study. If there had been no response by the time their child was 11 months old then they would receive a further invitation to participate.

Ethical approval was requested and granted for these amendments. The study literature was then updated to reflect the changes.

5.2.6 Summary

The main questions addressed by the pilot study were:
1. Will hospitals participate?
2. Will families participate?
3. Does the design need any modification?

It was clear that hospitals were willing to be involved, with all 12 hospitals in the Yorkshire Neonatal Network taking part, and an additional 29 hospitals taking part by the end of the pilot study period. Further, the estimated ascertainment for both the groups of hospitals – those within the Yorkshire Neonatal Network and those in other Neonatal Networks – was between two thirds and three quarters which indicated that families were willing to participate. Although, the logistical side of the study worked well on the whole, but review of the pilot period indicated the need for two significant refinements:
1. The location of the hospital laboratory processing the full blood counts moved to YH;
2. The study literature was amended
   a. to request permission to access the relevant maternity records in the newborn consent stage;
   b. to allow more flexibility over the timing of the move to the follow-up stage.
5.3 Hospital participation

Clinicians in each hospital with maternity services in the six Neonatal Networks which comprised the target area were identified and contacted in order to arrange a presentation about the study. Each hospital was then visited at least once and a presentation about the CDSS was made to the relevant team. Following the meetings a local study lead was appointed. Applications for Research and Development (R&D) approval were coordinated by the CDSS. These application procedures varied widely from hospital to hospital. Presentations about the study were also made to four separate Neonatal Network meetings at which the Neonatal Leads for each hospital within a given network met together.

All hospitals with maternity services in the six Neonatal Networks within the target area took part in the CDSS. Initially there were a total of 60 hospitals across the Neonatal Networks, but due to service reorganisation within the NHS several maternity units subsequently closed down. More recently the CDSS was approached by the West Midlands (South) Comprehensive Local Research Network (CLRN) who had identified the CDSS from the UKCRN Portfolio database. Following a presentation about the CDSS to clinicians from this CLRN the CDSS was established in three hospitals belonging to this network.

The details of hospital activity, including the number of registered births per annum for each hospital and the number of cases recruited by each hospital are provided in Table 5-3. Children were recruited from all of the 6 Neonatal Networks and from the West Midlands (South) CLRN. All of the hospitals within the Cheshire and Merseyside, Greater Manchester, Trent and Yorkshire Neonatal Networks recruited cases with the Yorkshire Neonatal Network being the most active and recruiting a total of 157 cases. There was one hospital in the North Trent and 4 hospitals in the Northern Neonatal Networks which did not recruit any cases. These tended to be hospitals where there was a relatively low birth rate. Only one of the three hospitals from the West Midlands (South) CLRN recruited cases into the CDSS.
Table 5-3. Participation in the CDSS by hospital and neonatal network.

<table>
<thead>
<tr>
<th>Location</th>
<th>Date of presentation</th>
<th>Date study opened</th>
<th>Registered births per a</th>
<th>Cases recruited to the CDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHESHIRE AND MERSEYSIDE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CM1</td>
<td>16/11/06</td>
<td>05/02/07</td>
<td>3515</td>
<td>12</td>
</tr>
<tr>
<td>CM2</td>
<td>16/11/06</td>
<td>24/04/07</td>
<td>3175</td>
<td>5</td>
</tr>
<tr>
<td>CM3</td>
<td>07/06/06</td>
<td>21/06/06</td>
<td>2807</td>
<td>6</td>
</tr>
<tr>
<td>CM4</td>
<td>23/08/06</td>
<td>26/10/06</td>
<td>8758</td>
<td>15</td>
</tr>
<tr>
<td>CM5</td>
<td>06/12/06</td>
<td>22/02/07</td>
<td>987</td>
<td>6</td>
</tr>
<tr>
<td>CM6</td>
<td>05/03/08</td>
<td>12/09/08</td>
<td>3132</td>
<td>1</td>
</tr>
<tr>
<td>CM7</td>
<td>09/08/06</td>
<td>16/11/06</td>
<td>3250</td>
<td>10</td>
</tr>
<tr>
<td>CM8</td>
<td>26/10/06</td>
<td>19/12/06</td>
<td>3078</td>
<td>3</td>
</tr>
<tr>
<td><strong>Network overall</strong></td>
<td><strong>09/06/06</strong></td>
<td><strong>-</strong></td>
<td><strong>28702</strong></td>
<td><strong>58</strong></td>
</tr>
<tr>
<td><strong>GREATER MANCHESTER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>23/01/07,20/11/07</td>
<td>12/03/07</td>
<td>3121</td>
<td>2</td>
</tr>
<tr>
<td>M2a</td>
<td>11/07/06,21/11/06</td>
<td>22/03/08</td>
<td>10 343&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>M2b</td>
<td>11/07/06,18/10/07</td>
<td>01/12/06</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>M2c</td>
<td>11/07/06,13/10/06</td>
<td>10/10/06</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>M2d</td>
<td>11/07/06,14/11/06</td>
<td>25/01/07</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>M3</td>
<td>12/12/06</td>
<td>12/03/07</td>
<td>3149</td>
<td>4</td>
</tr>
<tr>
<td>M4</td>
<td>10/07/06</td>
<td>18/09/06</td>
<td>5427</td>
<td>14</td>
</tr>
<tr>
<td>M5</td>
<td>12/10/06</td>
<td>21/11/6</td>
<td>3898</td>
<td>8</td>
</tr>
<tr>
<td>M6</td>
<td>06/12/07</td>
<td>26/02/08</td>
<td>3230</td>
<td>2</td>
</tr>
<tr>
<td>M7</td>
<td>07/11/06</td>
<td>22/12/06</td>
<td>4707</td>
<td>6</td>
</tr>
<tr>
<td>M8</td>
<td>05/06/06</td>
<td>07/08/06</td>
<td>1168</td>
<td>3</td>
</tr>
<tr>
<td>M9</td>
<td>24/10/07</td>
<td>21/03/08</td>
<td>3484</td>
<td>12</td>
</tr>
<tr>
<td><strong>Network overall</strong></td>
<td><strong>-</strong></td>
<td><strong>-</strong></td>
<td><strong>38527</strong></td>
<td><strong>62</strong></td>
</tr>
</tbody>
</table>

a. taken from www.hesonline.nhs.uk using 2009-2010 data
b. Hospitals M2a, M2b, M2c and M2d belong to the same Trust and these are the combined number of live births.
<table>
<thead>
<tr>
<th>Location</th>
<th>Date of presentation</th>
<th>Date study opened</th>
<th>Registered births pa$^a$</th>
<th>Cases recruited to the CDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORTH TRENT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT1</td>
<td>23/05/06</td>
<td>22/06/06</td>
<td>2987</td>
<td>4</td>
</tr>
<tr>
<td>NT2</td>
<td>16/01/07</td>
<td>14/06/07</td>
<td>1389</td>
<td>0</td>
</tr>
<tr>
<td>NT3</td>
<td>05/09/06</td>
<td>16/11/06</td>
<td>2877</td>
<td>8</td>
</tr>
<tr>
<td>NT4a</td>
<td>10/08/06</td>
<td>15/06/06</td>
<td>4697$^c$</td>
<td>7</td>
</tr>
<tr>
<td>NT4b</td>
<td>28/11/07</td>
<td>15/06/06</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>NT5</td>
<td>22/05/08</td>
<td>29/07/08</td>
<td>3859</td>
<td>1</td>
</tr>
<tr>
<td>NT6</td>
<td>13/12/07</td>
<td>25/08/06</td>
<td>6775</td>
<td>1</td>
</tr>
<tr>
<td>NT7</td>
<td>11/05/06</td>
<td>24/08/06</td>
<td>2778</td>
<td>11</td>
</tr>
<tr>
<td>Network overall</td>
<td>17/03/06</td>
<td>-</td>
<td>25362</td>
<td>37</td>
</tr>
<tr>
<td>NORTHERN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1a</td>
<td>13/09/06</td>
<td>08/01/07</td>
<td>6223$^d$</td>
<td>-</td>
</tr>
<tr>
<td>N1b</td>
<td>13/09/06</td>
<td>09/11/06</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>N1c</td>
<td>25/10/6</td>
<td>09/11/06</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>N2a</td>
<td>12/06/06</td>
<td>03/10/06</td>
<td>3021$^e$</td>
<td>4</td>
</tr>
<tr>
<td>N2b</td>
<td>30/11/06</td>
<td>03/10/06</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>N3</td>
<td>27/09/06</td>
<td>08/01/07</td>
<td>959</td>
<td>3</td>
</tr>
<tr>
<td>N4</td>
<td>27/09/06</td>
<td>08/01/07</td>
<td>4352</td>
<td>0</td>
</tr>
<tr>
<td>N5</td>
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<td>01/03/07</td>
<td>3055$^f$</td>
<td>0</td>
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<td>N6</td>
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<td>14/11/06</td>
<td>1929</td>
<td>3</td>
</tr>
<tr>
<td>N7</td>
<td>02/05/06</td>
<td>20/09/06</td>
<td>6683</td>
<td>16</td>
</tr>
<tr>
<td>N8</td>
<td>15/06/06</td>
<td>02/06/06</td>
<td>1564</td>
<td>4</td>
</tr>
<tr>
<td>N9</td>
<td>13/12/06</td>
<td>12/03/07</td>
<td>3377</td>
<td>12</td>
</tr>
<tr>
<td>N10a</td>
<td>07/08/06</td>
<td>14/11/06</td>
<td>3621$^g$</td>
<td>4</td>
</tr>
<tr>
<td>N10b</td>
<td>14/06/06</td>
<td>14/09/06</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Network overall</td>
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<td>-</td>
<td>34783</td>
<td>73</td>
</tr>
</tbody>
</table>

- Hospitals NT4a and NT4b belong to the same Trust and these are the combined number of live births.
- Hospitals N1a, N1b and N1c belong to the same Trust and these are the combined number of live births. No cases were recruited from N1a as the maternity unit closed.
- Hospitals N2a and N2b belong to the same Trust and these are the combined number of live births.
- There are several other maternity units associated with hospital N5, belonging to the same Trust, and all are included under this hospital.
- Hospitals N10a and N10b belong to the same Trust and these are the combined number of live births.
<table>
<thead>
<tr>
<th>Location</th>
<th>Date of presentation</th>
<th>Date study opened</th>
<th>Registered births pa$^a$</th>
<th>Cases recruited to the CDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRENT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>07/11/07</td>
<td>28/03/08</td>
<td>3641</td>
<td>1</td>
</tr>
<tr>
<td>T2</td>
<td>04/08/06</td>
<td>27/10/06</td>
<td>6012</td>
<td>16</td>
</tr>
<tr>
<td>T3</td>
<td>30/06/06</td>
<td>11/09/06</td>
<td>3033</td>
<td>6</td>
</tr>
<tr>
<td>T4a</td>
<td>28/06/06</td>
<td>23/08/06</td>
<td>5640</td>
<td>27</td>
</tr>
<tr>
<td>T4b</td>
<td>28/06/06</td>
<td>23/08/06</td>
<td>4560</td>
<td>13</td>
</tr>
<tr>
<td>T5</td>
<td>11/09/07</td>
<td>28/03/08</td>
<td>2223</td>
<td>5</td>
</tr>
<tr>
<td><strong>Network overall</strong></td>
<td>-</td>
<td>-</td>
<td><strong>25109</strong></td>
<td><strong>68</strong></td>
</tr>
<tr>
<td><strong>YORKSHIRE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y1</td>
<td>05/12/05</td>
<td>26/05/06</td>
<td>2511</td>
<td>8</td>
</tr>
<tr>
<td>Y2</td>
<td>08/12/05</td>
<td>26/06/06</td>
<td>6065</td>
<td>36</td>
</tr>
<tr>
<td>Y3a</td>
<td>18/08/06</td>
<td>03/05/06</td>
<td>5545$^h$</td>
<td>11</td>
</tr>
<tr>
<td>Y3b</td>
<td>11/11/05,18/08/06</td>
<td>03/05/06</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Y4a</td>
<td>18/11/05</td>
<td>11/05/06</td>
<td>3238</td>
<td>13</td>
</tr>
<tr>
<td>Y4b</td>
<td>07/02/06</td>
<td>11/05/06</td>
<td>3316</td>
<td>13</td>
</tr>
<tr>
<td>Y5</td>
<td>03/11/05</td>
<td>11/07/06</td>
<td>2293</td>
<td>5</td>
</tr>
<tr>
<td>Y6</td>
<td>07/12/05</td>
<td>14/06/06</td>
<td>5627</td>
<td>21</td>
</tr>
<tr>
<td>Y7a</td>
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<td>11/04/06</td>
<td>9397$^i$</td>
<td>20</td>
</tr>
<tr>
<td>Y7b</td>
<td>07/11/05</td>
<td>11/04/06</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Y8</td>
<td>28/11/05,17/12/07</td>
<td>26/06/06</td>
<td>1632</td>
<td>4</td>
</tr>
<tr>
<td>Y9</td>
<td>11/11/05</td>
<td>26/06/06</td>
<td>3276</td>
<td>9</td>
</tr>
<tr>
<td><strong>Network overall</strong></td>
<td>11/10/05</td>
<td>-</td>
<td><strong>42900</strong></td>
<td><strong>157</strong></td>
</tr>
<tr>
<td><strong>WEST MIDLANDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WM1</td>
<td>19/05/09</td>
<td>01/11/09</td>
<td>5605</td>
<td>0</td>
</tr>
<tr>
<td>WM2</td>
<td>19/05/09</td>
<td>01/10/09</td>
<td>2252</td>
<td>3</td>
</tr>
<tr>
<td>WM3</td>
<td>19/05/09</td>
<td>01/01/11</td>
<td>5571</td>
<td>0</td>
</tr>
<tr>
<td><strong>Network overall</strong></td>
<td>19/05/09</td>
<td>-</td>
<td><strong>15284</strong></td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>

h. Hospitals Y3a and Y3b belong to the same Trust and these are the combined number of live births.

i. Hospitals Y7a and Y7b belong to the same Trust and these are the combined number of live births.
5.4 Recruitment

Overall, 458 children were recruited into the CDSS by hospitals in the target area. The numbers recruited by each hospital and by each Neonatal Network are shown in Table 5-3. The numbers recruited into the CDSS from the start of the study are shown for each yearly quarter in Figure 5-1. Quarterly recruitment initially increased during each quarter as expected as the number of participating hospitals increased. Recruitment for the second quarter of 2007 was above 20 cases, and stayed above 20 for each quarter for the next three years. Recruitment was less than 20 for the second and third quarters of 2010, but this is balanced by a particularly high recruitment of over 30 cases for the last quarter of 2010. The cumulative recruitment is shown in Figure 5-2.

![Figure 5-1. Recruitment into the CDSS by quarter.](image-url)
Figure 5-2. Cumulative recruitment into the CDSS.

5.5 Case characteristics for the CDSS

The case characteristics for children entered into the CDSS are summarised in Table 5-4. Of the 479 children with newborn consent there were 234 (48.9%) with an eligible neonatal sample. There is no significant difference in the mean birth weight or gestational age for those with an eligible neonatal sample, those with an ineligible neonatal sample or those with no neonatal sample.
Table 5-4. Characteristics of the children entered in the CDSS.

<table>
<thead>
<tr>
<th></th>
<th>All cases with newborn consent</th>
<th>Cases with an eligible neonatal sample</th>
<th>Cases with an ineligible neonatal sample</th>
<th>Cases with no neonatal sample</th>
<th>Cases with a neonatal blood film</th>
<th>Cases with a sample at 0-3 months</th>
<th>Cases with a sample at 1st birthday</th>
<th>Cases with a sample at 2nd birthday</th>
<th>Cases with a sample at 3rd birthday</th>
<th>Cases with a sample at 4th birthday</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>479</td>
<td>234</td>
<td>27</td>
<td>218</td>
<td>189</td>
<td>247</td>
<td>48</td>
<td>10</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td><strong>Sex: boys / girls / missing</strong></td>
<td>265 (55%) / 213 (44%) / 1 (&lt;1%)</td>
<td>125 (53%) / 109 (47%) / 10 (37%)</td>
<td>17 (63%) / 94 (43%) / 94 (50%)</td>
<td>123 (56%) / 95 (50%) / 94 (50%)</td>
<td>134 (54%) / 113 (46%) / 24 (50%)</td>
<td>24 (50%) / 24 (50%) / 7 (70%)</td>
<td>12 (86%) / 3 (30%) / 12 (86%)</td>
<td>14 (92%) / 2 (14%) / 3 (60%)</td>
<td>5 (100%) / 3 (40%) / 2 (40%)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean birth weight (grams)</strong></td>
<td>2881</td>
<td>2878</td>
<td>2983</td>
<td>2871</td>
<td>2918</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Mean gestation (weeks)</strong></td>
<td>37.6</td>
<td>37.6</td>
<td>38</td>
<td>37.6</td>
<td>37.6</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Chapter 6  Results: haematological parameters in DS neonates.

6.1  Introduction

This chapter presents the results of the full blood count analysis for DS neonates in the CDSS. Values are expressed as mean (95% CI) and range (5- 95% percentiles). Where results are referred to as significant, this indicates that p<0.05.

6.2  Case characteristics

The case characteristics for neonates eligible for the analysis of haematological parameters are shown in Table 6-1.

Table 6-1. Case characteristics for samples included in the neonatal full blood count analysis.

<table>
<thead>
<tr>
<th></th>
<th>Sampled within 28 days of birth</th>
<th>Sampled within 7 days of birth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>234</td>
<td>(100)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>125</td>
<td>(53)</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2500</td>
<td>57</td>
<td>(24)</td>
</tr>
<tr>
<td>2501 to 3499</td>
<td>116</td>
<td>(50)</td>
</tr>
<tr>
<td>≥ 3500</td>
<td>28</td>
<td>(12)</td>
</tr>
<tr>
<td>missing</td>
<td>33</td>
<td>(14)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 to 32</td>
<td>10</td>
<td>(4)</td>
</tr>
<tr>
<td>33 to 37</td>
<td>77</td>
<td>(33)</td>
</tr>
<tr>
<td>38 to 42</td>
<td>140</td>
<td>(60)</td>
</tr>
<tr>
<td>missing</td>
<td>7</td>
<td>(3)</td>
</tr>
<tr>
<td>Processing time (days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>132</td>
<td>(56)</td>
</tr>
<tr>
<td>3-7</td>
<td>102</td>
<td>(44)</td>
</tr>
</tbody>
</table>

A total of 234 cases had a sample taken within 28 days of birth, and of these 177 samples were taken within 7 days of birth. There was a slight male preponderance, as there is in the general population, with 53% cases being male.

As described in the methods information about birth weight and gestational age was requested on the hospital referral form completed by the clinician recruiting the family, and also on the initial questionnaire sent out to families. Information about birth weight was missing for 33 (14%) cases, and for gestational age for 7 (3%) cases. Half of cases had a
birth weight between 2501 to 3499 grams, with 24% being under this, and 12% being over this. The distribution remained very similar when only those with a sample taken within 7 days of birth were considered. Just over half of all samples were processed within 2 days of venepuncture, with the remainder of samples being processed within 7 days of venepuncture. Again, this distribution was similar when only those with a sample taken within 7 days of birth were considered. Just over a third of cases were preterm, with 4% being born at 30-32 weeks gestation, 33% being born at 33-37 weeks gestation and 60% being born at 38-42 weeks gestation. When those with samples taken within 7 days of birth were considered there was a slight increase in the proportion born at term, with 65% cases being born at 38-42 weeks gestation.

6.3 Results for samples taken within the first week of life

This section considers samples taken within the first week of life only.

6.3.1 Red blood cells

These results are shown in Table 6-2. The red blood cell values for all samples were as follows: red blood cell count 5.6 (4.3-6.6) x10^{12} cells/l; haemoglobin 21.1 (16.8-25.3) g/dl; mean cell haemoglobin 37.4 (33.8-41.3) pg; haematocrit 0.6 (0.51-0.76) %; mean cell volume 115.5 (102.1-128.5) fl; and nucleated red blood cells 3.8 (0-20.3) x10^{9} cells/l. There was little difference when the sample set was restricted to only those processed within 2 days. The main changes were a slight fall in the haemoglobin to 20.9 (15.9-25.8) g/dl; and in the mean cell volume to 111.7 (98.2-123.4) fl.

6.3.2 White blood cell parameters

These results are shown in Table 6-3. The white blood cell values for all samples were as follows: white cell count 12.9 (6.1-24.2) x10^{9} cells/l; neutrophils 8 (1.9-16) x10^{9} cells/l; lymphocytes 3.6 (1.5-7.5) x10^{9} cells/l; basophils 0.2 (0-0.6) x10^{9} cells/l; eosinophils 0.2 (0-0.4) x10^{9} cells/l; and monocytes 1.0 (0.2-2.0) x10^{9} cells/l. There were no notable differences in these when the set was restricted to those processed within 2 days only.

These white blood cell results are restricted to those counts where the machine produced a complete white blood cell differential. In some cases the machine was not able to adequately assign a white blood cell subtype. When this occurred the machine was most likely to omit to give a neutrophil or lymphocyte count. In some of these cases a basophil count was the only white blood cell subtype to be obtained. The results from samples where a full differential count was available were compared with those from samples where only a part of the white
blood cell differential was obtained. This indicated that those with a full white blood cell
differential were the most robust. This consideration has some overlap with the time to
processing, which is considered later in this chapter, but is not the same.

6.3.3 Platelets

These results are shown in Table 6-4. The platelet count was 142 (35-255) x10^9 cells/l and
mean platelet volume 12.1 (10.4-13.9) fl for all samples. These only changed slightly when
the set was restricted to those processed within 2 days, yielding a marginally higher platelet
count of 149 (35-290) x10^9 cells/l and a lower mean platelet volume of 11.7 (9.8-13.5) fl.
Table 6-2. Range and mean values for red blood count parameters in DS neonates in the first week of life and regression analysis of gestation and birth weight.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range (5-95% percentile)</th>
<th>Mean count (95% CI)</th>
<th>Regression Model Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Model N**</td>
</tr>
<tr>
<td>N‡</td>
<td></td>
<td></td>
<td>Gestation (per added week)</td>
</tr>
<tr>
<td><strong>PROCESSED WITHIN 2 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Cell Count (x10^{12} cells/l)</td>
<td>95 4.09-6.62</td>
<td>5.6 (5.4, 5.7)</td>
<td>77 0.21 (0.1, 0.31)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>95 15.9-25.8</td>
<td>20.9 (20.2, 21.5)</td>
<td>77 0.78 (0.38, 1.18)</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin (pg)</td>
<td>95 33.6-41.2</td>
<td>37.4 (36.9, 37.9)</td>
<td>77 0.04 (-0.3, 0.38)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>94 0.46-0.74</td>
<td>0.6 (0.6, 0.6)</td>
<td>76 0.02 (0.01, 0.03)</td>
</tr>
<tr>
<td>Mean Cell Volume (fl)</td>
<td>95 98.2-123.4</td>
<td>111.7 (110.2, 113.2)</td>
<td>77 0.05 (-0.97, 1.07)</td>
</tr>
<tr>
<td>Nucleated RBC (x10^{9} cells/l)</td>
<td>73 0-22.8</td>
<td>4.2 (2.1, 6.3)</td>
<td>57 0.06 (-1.5, 1.62)</td>
</tr>
<tr>
<td><strong>PROCESSED WITHIN 7 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Cell Count (x10^{12} cells/l)</td>
<td>177 4.3-6.6</td>
<td>5.6 (5.5, 5.7)</td>
<td>150 0.15 (0.08, 0.22)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>177 16.8-25.3</td>
<td>21.1 (20.7, 21.5)</td>
<td>150 0.55 (0.29, 0.81)</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin (pg)</td>
<td>177 33.8-41.3</td>
<td>37.7 (37.3, 38)</td>
<td>150 -0.0024 (-0.24, 0.24)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>176 0.51-0.76</td>
<td>0.6 (0.6, 0.7)</td>
<td>149 0.02 (0.01, 0.02)</td>
</tr>
<tr>
<td>Mean Cell Volume (fl)</td>
<td>177 102.1-128.5</td>
<td>115.5 (114.3, 116.6)</td>
<td>150 0.12 (-0.69, 0.93)</td>
</tr>
<tr>
<td>Nucleated RBC (x10^{9} cells/l)</td>
<td>145 0-20.3</td>
<td>3.8 (2.5, 5.2)</td>
<td>122 0.18 (-0.79, 1.15)</td>
</tr>
</tbody>
</table>

Blood parameters significantly (p<0.05) affected by gestation or birth weight are shown in bold.
Table 6-3. Range and mean values for white blood count parameters in DS neonates in the first week of life and regression analysis of gestation and birth weight.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range (5-95% percentile)</th>
<th>Mean count (95% CI)</th>
<th>Regression Model Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N‡</td>
<td>Model N**</td>
<td>Gestation (per added week)</td>
</tr>
<tr>
<td><strong>PROCESSED WITHIN 2 DAYS: FULL DIFFERENTIAL COUNT AVAILABLE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Cell Count (x10^9 cells/l)</td>
<td>68 6.1-24.2</td>
<td>13.0 (11.2, 14.9)</td>
<td>56 0.47 (-0.71, 1.65)</td>
</tr>
<tr>
<td>Neutrophils (x10^9 cells/l)</td>
<td>68 1.9-18.5</td>
<td>8.1 (6.6, 9.5)</td>
<td>56 0.69 (-0.21, 1.58)</td>
</tr>
<tr>
<td>Lymphocytes (x10^9 cells/l)</td>
<td>68 1.5-7.5</td>
<td>3.6 (3.1, 4.1)</td>
<td>56 -0.09 (-0.43, 0.25)</td>
</tr>
<tr>
<td>Basophils (x10^9 cells/l)</td>
<td>68 0-0.6</td>
<td>0.2 (0.1, 0.3)</td>
<td>56 -0.0033 (-0.03, 0.02)</td>
</tr>
<tr>
<td>Eosinophils (x10^9 cells/l)</td>
<td>68 0-0.5</td>
<td>0.2 (0.1, 0.2)</td>
<td>56 0.0039 (-0.02, 0.03)</td>
</tr>
<tr>
<td>Monocytes (x10^9 cells/l)</td>
<td>68 0.3-2.1</td>
<td>1.0 (0.9, 1.2)</td>
<td>56 -0.023 (-0.11, 0.06)</td>
</tr>
<tr>
<td><strong>PROCESSED WITHIN 7 DAYS: FULL DIFFERENTIAL COUNT AVAILABLE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Cell Count (x10^9 cells/l)</td>
<td>81 6.1-24.2</td>
<td>12.9 (11.3, 14.6)</td>
<td>67 0.39 (-0.597, 1.38)</td>
</tr>
<tr>
<td>Neutrophils (x10^9 cells/l)</td>
<td>81 1.9-16.0</td>
<td>8.0 (6.7, 9.3)</td>
<td>67 0.58 (-0.176, 1.33)</td>
</tr>
<tr>
<td>Lymphocytes (x10^9 cells/l)</td>
<td>81 1.5-7.5</td>
<td>3.6 (3.1, 4.1)</td>
<td>67 -0.05 (-0.337, 0.24)</td>
</tr>
<tr>
<td>Basophils (x10^9 cells/l)</td>
<td>81 0-0.6</td>
<td>0.2 (0.1, 0.2)</td>
<td>67 -0.005 (-0.027, 0.02)</td>
</tr>
<tr>
<td>Eosinophils (x10^9 cells/l)</td>
<td>81 0-0.4</td>
<td>0.2 (0.1, 0.2)</td>
<td>67 0.01 (-0.011, 0.03)</td>
</tr>
<tr>
<td>Monocytes (x10^9 cells/l)</td>
<td>81 0.3-2.0</td>
<td>1.0 (0.9, 1.1)</td>
<td>67 -0.03 (-0.111, 0.05)</td>
</tr>
</tbody>
</table>
Table 6.4. Range and mean values for platelet parameters in DS neonates in the first week of life and regression analysis of gestation and birth weight for samples.

<table>
<thead>
<tr>
<th></th>
<th>Range (5-95% percentile)</th>
<th>Mean count (95% CI)</th>
<th>Regression Model Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N‡</td>
<td>Model</td>
<td>Gestation (per added week)</td>
</tr>
<tr>
<td><strong>PROCESSED WITHIN 2 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Count (x10^9 cells/l)</td>
<td>93</td>
<td>35-290</td>
<td>149 (133.2, 164.2)</td>
</tr>
<tr>
<td>Mean Platelet Volume (fl)</td>
<td>27</td>
<td>9.8-13.5</td>
<td>11.7 (11.3, 12.2)</td>
</tr>
<tr>
<td><strong>PROCESSED WITHIN 7 DAYS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Count (x10^9 cells/l)</td>
<td>172</td>
<td>35-255</td>
<td>142 (131.9, 152.1)</td>
</tr>
<tr>
<td>Mean Platelet Volume (fl)</td>
<td>50</td>
<td>10.4-13.9</td>
<td>12.1 (11.8, 12.4)</td>
</tr>
</tbody>
</table>

Blood parameters significantly (p<0.05) affected by gestation or birth weight are shown in bold.
6.4 Association with birth weight

As this is likely to be most relevant to samples taken closer to birth this was examined in cases with samples taken within the first 7 days of life. The results for all red blood cell, white blood cell and platelet parameters are shown in Table 6-2, Table 6-3 and Table 6-4 respectively. Where there is a significant result this is highlighted in bold.

Red blood cells

Despite the association between birth weight and gestational age, there were no significant association between birth weight and red blood cell parameters.

White blood cells

There was no significant association between birth weight and white blood cell parameters.

Platelets

Regression analysis showed that there was a small increase in the platelet count with increasing birth weight. This remained significant (p<0.05) and the association became larger when the set was restricted to those processed within 2 days, with an increase in platelet count of 6.5 (3.3, 9.8) x10^9 cells/l for each 100g increase in birth weight. This is shown in Figure 6-1.

![Figure 6-1. The association between birth weight and platelet count for neonates with DS sampled within the first seven days of life.](image)

Predicted values (+/- 95% CI) from linear regression models fitted to the data are shown. Age at sampling and gestational age were set to 2 days of age and 38 weeks, respectively, to generate the predictions. All measures were based on samples processed within seven days of sampling.
6.5 Association with gestational age

Again this was examined in cases with samples taken within the first 7 days of life. The results for all red blood cell, white blood cell and platelet parameters are shown in Table 6-2, Table 6-3 and Table 6-4 respectively. Where there is a significant result this is highlighted in bold.

Red blood cells

Increasing gestational age was significantly associated (p<0.05) with an increase in haemoglobin, red cell count and haematocrit. This association remained and became slightly more pronounced when only those processed within 2 days were considered. In this set there was an increase in the haemoglobin of 0.78 g/dl, in the red cell count of 0.21 x10^{12} cells/l, and in the haematocrit of 0.02% for each added week of gestation. This is shown in Figure 6-2.

White blood cells

There was no significant association between gestational age and white blood cell parameters.

Platelets

Regression analysis showed that the platelet count fell with increasing gestational age. This remained significant (p<0.05) and the association became larger when the set was restricted to those processed within 2 days, with a fall in platelet count of 30.9 (40.1, 21.8) x10^9 cells/l for each added week of gestational age. This is shown in Figure 6-3. When samples processed within 7 days of sampling were considered the mean platelet volume appeared to increase with increasing gestational age. This association disappeared when the set was restricted to samples processed within 2 days only.
Figure 6-2. The association between gestational age and haemoglobin, haematocrit and red blood cell count in neonates with DS sampled within the first seven days of life.

Predicted values (+/- 95% CI) from linear regression models fitted to the data are shown in blue. Age at sampling and birth weight were set to 2 days of age and 3000 grams, respectively.
Figure 6-3. The association between gestational age and platelet count in neonates with DS sampled within the first seven days of life.

Predicted values (± 95% CI) from linear regression models fitted to the data are shown in blue. Age at sampling and birth weight were set to 2 days of age and 3000 grams, respectively.
6.6 Association with sampling to processing interval

This is the interval between taking the sample and sample processing, given in whole days. Data from all 234 children sampled with 28 days of birth were included in this analysis. All samples were processed within 7 days of venepuncture. The distribution was skewed so that more children had samples processed with a shorter interval than with a longer interval. Over half (56%) of samples were processed within 2 days of venepuncture. This is illustrated in Figure 6-4.

![Figure 6-4. Number of samples at each sampling to processing interval.](image)
6.6.1 Red blood cells

Irrespective of interval length, nearly all samples had a complete set of red cell measurements. The only exception was in the nucleated red blood cell count, which was missing in 34 cases. There was no apparent trend in the number of samples which had a missing nucleated red blood cell result with interval length.

The results are shown in Table 6-5. The mean red blood cell count, the haemoglobin, the mean cell haemoglobin and the nucleated red blood cell count did not vary significantly with increasing sampling to processing interval. The haematocrit and the mean cell volume both increased significantly (p<0.05) as the sampling to processing interval increased. The haematocrit increased by 0.013 % with each additional day interval between sampling and processing, and the mean cell volume increased by 2.117 fl for each additional day. This is shown graphically in Figure 6-5 and Figure 6-6. It can be seen that there is a trend for the mean values at each day interval to increase for the haematocrit and the mean cell volume. In contrast, the other lines are approximately linear.
Table 6-5. Association between the number of days between sampling and processing on all of the blood parameters from children sampled within 28 days of life.

<table>
<thead>
<tr>
<th></th>
<th>Number of children</th>
<th>Effect estimate (per day)</th>
<th>(95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RED BLOOD CELL PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Cell Count (x10^{12} cells/l)</td>
<td>234</td>
<td>0.008</td>
<td>(-0.048, 0.065)</td>
<td>0.77</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>234</td>
<td>0.106</td>
<td>(-0.118, 0.33)</td>
<td>0.36</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin (pg)</td>
<td>234</td>
<td>0.151</td>
<td>(-0.02, 0.322)</td>
<td>0.08</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>231</td>
<td>0.013</td>
<td>(0.007, 0.02)</td>
<td>6.00x10^{-5}</td>
</tr>
<tr>
<td>Mean Cell Volume (fl)</td>
<td>234</td>
<td>2.117</td>
<td>(1.614, 2.621)</td>
<td>1.79x10^{-16}</td>
</tr>
<tr>
<td>Nucleated RBC (x10^9 cells/l)</td>
<td>188</td>
<td>-0.388</td>
<td>(-0.951, 0.176)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

| **WHITE BLOOD CELL PARAMETERS: ALL SAMPLES PROCESSED WITHIN 7 DAYS** |                    |                           |                |         |
| White Cell Count (x10^9 cells/l) | 234                | 0.036                     | (-0.536, 0.609) | 0.90    |
| Neutrophils (x10^9 cells/l)     | 125                | -0.722                    | (-1.335, -0.109) | 2.10E-02 |
| Lymphocytes (x10^9 cells/l)     | 126                | -0.197                    | (-0.474, 0.08)  | 0.16    |
| Basophils (x10^9 cells/l)       | 203                | 0.005                     | (-0.009, 0.019) | 0.50    |
| Eosinophils (x10^9 cells/l)     | 137                | -0.008                    | (-0.023, 0.007) | 0.29    |
| Monocytes (x10^9 cells/l)       | 137                | -0.09                     | (-0.144, -0.035) | 0.00    |

| **WHITE BLOOD CELL PARAMETERS: FULL DIFFERENTIAL; PROCESSED WITHIN 7 DAYS** |                    |                           |                |         |
| White Cell Count (x10^9 cells/l) | 125                | -0.926                    | (-1.735, -0.117) | 0.02    |
| Neutrophils (x10^9 cells/l)     | 125                | -0.722                    | (-1.335, -0.109) | 2.10E-02 |
| Lymphocytes (x10^9 cells/l)     | 125                | -0.152                    | (-0.438, 0.134) | 2.96E-01 |
| Basophils (x10^9 cells/l)       | 125                | -0.003                    | (-0.025, 0.019) | 7.97E-01 |
| Eosinophils (x10^9 cells/l)     | 125                | -0.005                    | (-0.021, 0.011) | 5.21E-01 |
| Monocytes (x10^9 cells/l)       | 125                | -0.075                    | (-0.136, -0.014) | 1.62E-02 |

| **WHITE BLOOD CELL PARAMETERS: FULL DIFFERENTIAL; PROCESSED WITHIN 2 DAYS** |                    |                           |                |         |
| White Cell Count (x10^9 cells/l) | 101                | -2.769                    | (-4.58, -0.958) | 2.73E-03 |
| Neutrophils (x10^9 cells/l)     | 101                | -2.127                    | (-3.525, -0.729) | 2.86E-03 |
| Lymphocytes (x10^9 cells/l)     | 101                | -0.544                    | (-1.162, 0.074) | 8.45E-02 |
| Basophils (x10^9 cells/l)       | 101                | 0.021                     | (-0.03, 0.073)  | 4.19E-01 |
| Eosinophils (x10^9 cells/l)     | 101                | 0.016                     | (-0.02, 0.051)  | 3.90E-01 |
| Monocytes (x10^9 cells/l)       | 101                | 0.084                     | (-0.052, 0.22)  | 2.25E-01 |

| **PLATELET PARAMETERS** |                    |                           |                |         |
| Platelet Count (x10^9 cells/l) | 227                | -4.937                    | (-11.069, 1.194) | 0.11    |
| Mean Platelet Volume (fl)     | 79                 | 0.195                     | (0.058, 0.332)  | 5.32x10^{-3} |

Blood parameters significantly (P<0.05) affected by the number of days between sampling and processing are shown in bold.
Figure 6.5. Stability of haemoglobin, haematocrit and red cell count with increasing sampling to processing interval.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval. An increasing trend is apparent for haematocrit; the other lines are approximately linear.
Figure 6-6. Stability of mean cell haemoglobin, mean cell volume and nucleated red cell count with increasing sampling to processing interval.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval. An increasing trend is apparent for mean cell volume; the other lines are approximately linear.
6.6.2 White blood cells

The total white cell count was reported for all cases, regardless of interval length. However, a differential white cell count was not reported for all of these. A sample was less likely to have a differential white cell count with each increase in interval time. Once the sampling to processing interval reached ≥3 days fewer than 50% of the samples had a differential white cell count.

In order to examine the effect of interval length more closely the white blood cell data was considered in three sets: all samples; samples processed within 7 days and with a complete differential white blood cell count; and samples processed within 2 days and with a complete differential white blood cell count. These results are shown in Table 6-5.

In all three sets the neutrophil count fell with increasing interval length. The neutrophil count accounts for the majority of the total white cell count. The total white blood cell count fell with increasing sampling to processing interval length in the two sets which were restricted to those samples with a complete white blood cell differential. However, this effect is lost in the set that included all samples. Taken together the data indicates that the total white cell count and the neutrophil count both decrease with increasing interval length. The monocyte count also fell with increasing interval length.

The change in white blood cell parameters with increasing sampling to processing interval is shown graphically in Figure 6-7 and Figure 6-8. When the set is restricted to samples processed within 2 days only then the lines between the means for lymphocytes, monocytes, basophils and eosinophils are all approximately linear. However, the mean neutrophil count clearly falls after a sampling to processing interval of one day indicating that this parameter is the least stable and also showing that this drives the results for total white cell count stability.
Figure 6-7. Stability of white blood cell, neutrophil and lymphocyte counts with increasing sampling to processing interval.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval. When a 2 day cut-off is used it can be seen that the lymphocyte results are approximately linear. However, the neutrophil count clearly falls in the first 24 hour interval.
Figure 6-8. Stability of monocyte, basophil and eosinophil counts with increasing sampling to processing interval.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval. When a 2 day cut-off is used it can be seen that the monocyte, basophil and eosinophil results are approximately linear.
6.6.3 Platelets

A platelet count was recorded in 227/234 samples. In some cases it was not possible to obtain a platelet count as there was a clot in the sample. There was no apparent relationship between the likelihood of having either a platelet count or mean platelet volume reported and sampling to processing interval length. Mean platelet volume was reported in a minority of the samples.

The results of the regression analysis for the stability with increasing sampling to processing interval are provided in Table 6-5. Mean platelet volume increased by 0.195 fl with each additional day between sampling and processing. There was only one observation at seven days post-sampling which may have influenced the fit of the model. The change in platelet parameters with increasing sampling to processing interval is illustrated in Figure 6-9. There is an upward trend in the mean result for mean platelet volume after a sampling to processing interval of 5 days, although there are fewer data points with higher sampling to processing intervals which may distort this. When a cut-off of 4 days is used it can be seen that the line becomes approximately linear.
Figure 6-9. Stability of platelet parameters with increasing sampling to processing interval.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval. An increasing trend is apparent for the mean platelet volume (B), although the sharp increase between 6 and 7 days is likely to be exaggerated as there is only one result for day 7. When a cut-off of 4 days is used the line is approximately linear.
6.6.4 Determining the best cut-off for reliability

Overall, there were six parameters that varied significantly (p<0.05) with an increasing sampling to processing interval length within the 7 day period: haematocrit; mean red blood cell volume; white blood cell count; neutrophil count; monocyte count; and mean platelet volume. The model used to examine this had compared results with a sampling to processing interval of 0 (ie processed on the day of sampling) with those from samples with a sampling to processing interval of ≤7 days. The analysis showed that results for these six parameters derived from samples with a sampling to processing interval of ≤7 days were significantly (p<0.05) different from those just taken from samples processed on the day of sampling. However, for all other parameters there was no significant difference in results with the sampling to processing interval and the parameters are demonstrated to be stable.

In order to determine when the sampling to processing interval became important for each of these six parameters further regression analysis models were created for each of the six parameters. In these models the results from samples processed on the day of sampling were compared with results from all samples with a sampling to processing interval of ≤1 day, ≤2 days, ≤3 days, ≤4 days, ≤5 days and ≤6 days. The longest sampling to processing interval for which there was no significant (p<0.05) trend compared with the results from samples processed on the day of sampling was identified for each of the parameters. The data could then be restricted appropriately.

For all of the parameters apart from the six that varied with sampling a cut-off of 7 days could be used. The trend in mean platelet volume was removed with a cut-off point of 4 days indicating that results were acceptable up to and including those with a sampling to processing interval of ≤4 days. The trend in haematocrit, mean red blood cell volume and monocyte count disappeared when the cut-off point for these was reduced to ≤2 days indicating that results were acceptable up to and including those with a sampling to processing interval of ≤2 days. This is shown graphically in Figure 6-5, Figure 6-6 and Figure 6-8.

However, even when a cut-off of 2 days was used for the neutrophil count the regression model showed a significant difference between results from samples processed on the day of sampling and those processed ≤2 days from sampling. This is shown in Figure 6-7.
6.7 Association with postnatal age

The association with postnatal age was examined in all of the 234 samples taken during the first 28 days of life. In addition, the mean value and 95% CI for each haematological parameter was calculated for each week of life during the neonatal period. These results are shown in Table 6-6, Table 6-7 and Table 6-8.

Table 6-6. Association with postnatal age and blood parameters for children with DS sampled within 28 days of life.

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>Number of children</th>
<th>Effect estimate (per day)</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RED BLOOD CELL PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Cell Count (x10^{12} cells/l)</td>
<td>234</td>
<td>-0.0391</td>
<td>(-0.0642, -0.014)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>234</td>
<td>-0.29</td>
<td>(-0.34, -0.23)</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin (pg)</td>
<td>234</td>
<td>-0.12</td>
<td>(-0.17, -0.07)</td>
</tr>
<tr>
<td>Haematocrit (%)^a</td>
<td>130</td>
<td>-0.0081</td>
<td>(-0.0106, -0.0056)</td>
</tr>
<tr>
<td>Mean Cell Volume (fl)^a</td>
<td>132</td>
<td>-0.15</td>
<td>(-0.37, 0.06)</td>
</tr>
<tr>
<td>Nucleated RBC (x10^9 cells/l)</td>
<td>200</td>
<td>-0.59</td>
<td>(-0.84, -0.34)</td>
</tr>
<tr>
<td><strong>WHITE BLOOD CELL PARAMETERS^a</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Cell Count (x10^9 cells/l)</td>
<td>101</td>
<td>-0.57937</td>
<td>(-0.89, -0.26)</td>
</tr>
<tr>
<td>Neutrophils (x10^9 cells/l)</td>
<td>101</td>
<td>-0.61</td>
<td>(-0.84, -0.39)</td>
</tr>
<tr>
<td>Lymphocytes (x10^9 cells/l)</td>
<td>101</td>
<td>0.018</td>
<td>(-0.053, 0.089)</td>
</tr>
<tr>
<td>Basophils (x10^9 cells/l)</td>
<td>101</td>
<td>-0.0047</td>
<td>(-0.0105, 0.0011)</td>
</tr>
<tr>
<td>Eosinophils (x10^9 cells/l)</td>
<td>101</td>
<td>0.0003</td>
<td>(-0.0038, 0.0043)</td>
</tr>
<tr>
<td>Monocytes (x10^9 cells/l)</td>
<td>101</td>
<td>0.0034</td>
<td>(-0.0121, 0.0189)</td>
</tr>
<tr>
<td><strong>PLATELET PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Count (x10^9 cells/l)</td>
<td>227</td>
<td>7.39</td>
<td>(5.86, 8.92)</td>
</tr>
<tr>
<td>Mean Platelet Volume (fl)^b</td>
<td>70</td>
<td>0.05</td>
<td>(0.00228, 0.09)</td>
</tr>
</tbody>
</table>

a. data restricted to samples processed within 2 days of venepuncture
b. data restricted to samples processed within 4 days of venepuncture

Blood parameters with a significant (p<0.05) association with postnatal age are shown in bold.
Table 6-7. Mean values for haematological parameters in children with DS sampled within 28 days of life shown for each week of life.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0-7 days</th>
<th>8-14 days</th>
<th>15-21 days</th>
<th>22-28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (95% CI)</td>
<td>N</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td><strong>RED BLOOD CELL PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Cell Count (x10^{12} cells/l)</td>
<td>177</td>
<td>5.6 (5.5, 5.7)</td>
<td>12</td>
<td>5.4 (5.2, 5.6)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>177</td>
<td>21.1 (20.7, 21.5)</td>
<td>12</td>
<td>19.8 (19.1, 20.5)</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin (pg)</td>
<td>177</td>
<td>37.7 (37.3, 38.0)</td>
<td>12</td>
<td>36.8 (36.3, 37.4)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>176</td>
<td>0.6 (0.6, 0.7)</td>
<td>12</td>
<td>0.61 (0.59, 0.63)</td>
</tr>
<tr>
<td>Mean Cell Volume (fl)</td>
<td>177</td>
<td>115.5 (114.3, 116.6)</td>
<td>12</td>
<td>113.3 (111.4, 115.2)</td>
</tr>
<tr>
<td>Nucleated RBC (x10^9 cells/l)</td>
<td>145</td>
<td>3.8 (2.5, 5.2)</td>
<td>12</td>
<td>0.68 (0.12, 1.24)</td>
</tr>
<tr>
<td><strong>WHITE BLOOD CELL PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Cell Count (x10^9 cells/l)</td>
<td>81</td>
<td>12.9 (11.3, 14.6)</td>
<td>8</td>
<td>9.9 (8.0, 11.7)</td>
</tr>
<tr>
<td>Neutrophils (x10^9 cells/l)</td>
<td>81</td>
<td>8.0 (6.7, 9.3)</td>
<td>8</td>
<td>4.6 (3.6, 5.5)</td>
</tr>
<tr>
<td>Lymphocytes (x10^9 cells/l)</td>
<td>81</td>
<td>3.6 (3.1, 4.1)</td>
<td>8</td>
<td>3.9 (2.9, 4.8)</td>
</tr>
<tr>
<td>Basophils (x10^9 cells/l)</td>
<td>81</td>
<td>0.2 (0.1, 0.2)</td>
<td>8</td>
<td>0.16 (0.13, 0.19)</td>
</tr>
<tr>
<td>Eosinophils (x10^9 cells/l)</td>
<td>81</td>
<td>0.2 (0.1, 0.2)</td>
<td>8</td>
<td>0.16 (0.12, 0.20)</td>
</tr>
<tr>
<td>Monocytes (x10^9 cells/l)</td>
<td>81</td>
<td>1.0 (0.9, 1.1)</td>
<td>8</td>
<td>1.0 (0.9, 1.2)</td>
</tr>
<tr>
<td><strong>PLATELET PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Count (x10^9 cells/l)</td>
<td>172</td>
<td>142 (131.9, 152.1)</td>
<td>11</td>
<td>164 (145, 184)</td>
</tr>
<tr>
<td>Mean Platelet Volume (fl)</td>
<td>50</td>
<td>12.1 (11.8, 12.4)</td>
<td>4</td>
<td>12.4 (11.8, 13.0)</td>
</tr>
</tbody>
</table>
Table 6-8. Ranges for haematological parameters in children with DS sampled within 28 days of life shown for each week of life.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0-7 days</th>
<th>8-14 days</th>
<th>15-21 days</th>
<th>22-28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Range (95% percentile)</td>
<td>N</td>
<td>Range (95% percentile)</td>
</tr>
<tr>
<td><strong>RED BLOOD CELL PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Cell Count (x10^{12} cells/l)</td>
<td>177</td>
<td>4.3-6.6</td>
<td>12</td>
<td>3.1-6.2</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>177</td>
<td>16.8-25.3</td>
<td>12</td>
<td>9.5-19.6</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin (pg)</td>
<td>177</td>
<td>33.8-41.3</td>
<td>12</td>
<td>28.6-39.5</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>176</td>
<td>0.51-0.76</td>
<td>12</td>
<td>0.3-0.69</td>
</tr>
<tr>
<td>Mean Cell Volume (fl)</td>
<td>177</td>
<td>102.1-128.5</td>
<td>12</td>
<td>84.6-127.1</td>
</tr>
<tr>
<td>Nucleated RBC (x10^{9} cells/l)</td>
<td>145</td>
<td>0-20.3</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td><strong>WHITE BLOOD CELL PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Cell Count (x10^{9} cells/l)</td>
<td>81</td>
<td>6.1-24.2</td>
<td>8</td>
<td>8.1-10.7</td>
</tr>
<tr>
<td>Neutrophils (x10^{9} cells/l)</td>
<td>81</td>
<td>1.9-16.0</td>
<td>8</td>
<td>2.2-4.8</td>
</tr>
<tr>
<td>Lymphocytes (x10^{9} cells/l)</td>
<td>81</td>
<td>1.5-7.5</td>
<td>8</td>
<td>3.4-4.9</td>
</tr>
<tr>
<td>Basophils (x10^{9} cells/l)</td>
<td>81</td>
<td>0-0.6</td>
<td>8</td>
<td>0.08-0.19</td>
</tr>
<tr>
<td>Eosinophils (x10^{9} cells/l)</td>
<td>81</td>
<td>0-0.4</td>
<td>8</td>
<td>0.01-0.31</td>
</tr>
<tr>
<td>Monocytes (x10^{9} cells/l)</td>
<td>81</td>
<td>0.3-2.0</td>
<td>8</td>
<td>0.6-2.7</td>
</tr>
<tr>
<td><strong>PLATELET PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Count (x10^{9} cells/l)</td>
<td>172</td>
<td>35-255</td>
<td>11</td>
<td>102-463</td>
</tr>
<tr>
<td>Mean Platelet Volume (fl)</td>
<td>50</td>
<td>10.4-13.9</td>
<td>4</td>
<td>10.2-12.6</td>
</tr>
</tbody>
</table>
Red blood cells

The haemoglobin, red blood cell count, haematocrit, mean cell haemoglobin and the nucleated red blood cell count all fell significantly \((p<0.05)\) during the neonatal period with increasing postnatal age. The haemoglobin fell by 0.0391 g/dl; the red blood cell count fell by \(0.29 \times 10^{12}\) cells/l; the haematocrit fell by 0.0081\%; the mean cell haemoglobin fell by 0.12 pg; and the nucleated red blood cell count fell by \(0.59 \times 10^{9}\) cells/l with each postnatal day. The nucleated red blood cell count fell to zero so that there were no nucleated red blood cells reported on samples \(\geq 9\) postnatal days old at sampling. The mean red blood cell volume remained fairly stable throughout the neonatal period. The pattern of change of the red blood cell parameters with postnatal age is illustrated in Figure 6-10 and Figure 6-11.

Children were considered polycythaemic if either the total haemoglobin was greater than 22 g/dl or the haematocrit was greater than 0.65. In the first week of life 95 (54\%) of the 176 cases were polycythaemic. This fell rapidly so that there were no cases of polycythaemia by the fourth week of life. Details are shown in Table 6-9.

<table>
<thead>
<tr>
<th>Age at Sampling (days)</th>
<th>Polycythaemic</th>
<th>(n)</th>
<th>Thrombocytopenic</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7</td>
<td>54</td>
<td>(176)</td>
<td>61</td>
<td>(172)</td>
</tr>
<tr>
<td>8-14</td>
<td>20</td>
<td>(35)</td>
<td>26</td>
<td>(35)</td>
</tr>
<tr>
<td>15-21</td>
<td>17</td>
<td>(12)</td>
<td>18</td>
<td>(11)</td>
</tr>
<tr>
<td>22-28</td>
<td>0</td>
<td>(8)</td>
<td>0</td>
<td>(9)</td>
</tr>
</tbody>
</table>

Table 6-9. Percentage of children who were polycythaemic or thrombocytopenic by age for each postnatal week.

Polycythaemia was defined as total haemoglobin >22 g/dl or haematocrit>0.65. Thrombocytopenia was defined as platelet count<150x109 cells/l.
Figure 6-10. The association between postnatal age and haemoglobin, haematocrit and red blood cell count in children with DS sampled within the first 28 days of life.

The black line shows the mean value with the 95% CI shown in blue.
Figure 6-11. The association between postnatal age and mean cell haemoglobin, mean cell volume and nucleated red blood cell count in children with DS sampled within the first 28 days of life.

The black line shows the mean value with the 95% CI shown in blue.
White blood cells

The association between postnatal age and white blood cell parameters is shown in Table 6-6. The white blood cell count fell by $1.026 \times 10^9$ cells/l; and the neutrophil count fell by $0.61 \times 10^9$ cells/l with each postnatal day. The pattern of this fall is illustrated in Figure 6-12. This shows that both the white blood cell count and the neutrophil count fall most rapidly during the first 2 weeks of life and are fairly stable thereafter. In the graphic model the count appears to rise towards the end of the neonatal period, but there are fewer data points at this end. The effect on the total white blood cell count is largely driven by the pattern of change of the neutrophil count. The only other white blood cell type to vary during the neonatal period is the basophil count which appears to fall throughout the neonatal period, but in a more linear fashion. This did not reach significance. The pattern of change of the lymphocyte, monocyte, basophil and eosinophil counts with increasing postnatal age is illustrated in Figure 6-12 and Figure 6-13.
Figure 6-12. The association between postnatal age and total white cell, neutrophil and lymphocyte count in children with DS sampled within the first 28 days of life.

The black line shows the mean value with the 95% CI shown in blue.
Figure 6-13. The association between postnatal age and monocyte, basophil and eosinophil count in children with DS sampled within the first 28 days of life.

The black line shows the mean value with the 95% CI shown in blue.
Platelets

The association between postnatal age and white blood cell parameters is shown in Table 6-6 and in Figure 6-14. In contrast to the red and white blood cell parameters, the platelet count increases in a linear manner throughout the neonatal period. The platelet count increased by $7.39 \times 10^9$ cells/l with each postnatal day. There was also an initial increase in the mean platelet volume, but this appeared to peak around the middle of the neonatal period and then decrease.

A child is considered to be thrombocytopenic if their platelet count is less than $150 \times 10^9$ cells/l. In the first week of life, 105 (61%) of the 172 cases were thrombocytopenic. This fell rapidly so that there were no cases of thrombocytopenia by the fourth week of life. Details are shown in Table 6-9.

![Figure 6-14. The association between postnatal age and platelet count and mean platelet volume in children with DS sampled within the first 28 days of life.](image)

The black line shows the mean value with the 95% CI shown in blue.
Chapter 7 Results: the blood cell morphology of neonates with DS

7.1 Introduction

This chapter presents the results of the neonatal blood cell morphology review for DS neonates within the CDSS using the morphology review tool developed for this research.

7.2 Case characteristics

The case characteristics are provided in Table 7-1.

Table 7-1. Case characteristics for samples included in the neonatal morphology analysis.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Suitable for full morphology review</th>
<th>Suitable for limited morphology review</th>
<th>Unsuitable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>189</td>
<td>51</td>
<td>72</td>
<td>66</td>
</tr>
<tr>
<td>Sex (number; %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>95</td>
<td>(50)</td>
<td>28</td>
<td>36</td>
</tr>
<tr>
<td>Female</td>
<td>94</td>
<td>(50)</td>
<td>23</td>
<td>36</td>
</tr>
<tr>
<td>Blood source (number; %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillary</td>
<td>32</td>
<td>(17)</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Venous</td>
<td>113</td>
<td>(60)</td>
<td>22</td>
<td>54</td>
</tr>
<tr>
<td>Unknown</td>
<td>44</td>
<td>(23)</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>Mean birth weight (grams)</td>
<td>2918</td>
<td>2796</td>
<td>2939</td>
<td>2982</td>
</tr>
<tr>
<td>Mean gestational age (weeks)</td>
<td>37.6</td>
<td>36.9</td>
<td>37.6</td>
<td>38.3</td>
</tr>
<tr>
<td>Age at sampling (days)</td>
<td>6.0</td>
<td>9.2</td>
<td>4.9</td>
<td>4.8</td>
</tr>
</tbody>
</table>

A stained slide was available for review in 189 cases. Of these, 51 (27%) met the criteria for a full morphology review according to the morphology review tool (Appendix 9) while 72 (38%) were suitable for a partial morphology review. A further 66 (35%) slides were not suitable for morphology review as a result of degenerative change as a consequence of the delay between sampling and processing. There were equal numbers of males and females. Most of the samples (60%) came from venepuncture. Just under a fifth (17%) were capillary samples and the blood source was unknown in just under a quarter (23%) of cases. When
this was broken down according to the suitability of the slide the proportion of capillary samples appears to increase in those with unsuitable samples.

There were no significant (p<0.05) differences in birth weight or gestational age between the groups with slides that were suitable for a full review, suitable for a partial review or unsuitable for morphological review. The mean birth weight for all cases in this analysis was 2918 grams. The mean birth weight was lower in the group with slides that were suitable for assessment, in keeping with the lower gestational age in this group. The mean gestational age for all cases in this analysis was 37.6 weeks. The mean age at sampling was 6 days. The mean age in the group with suitable samples was higher at 9.2 days, while the mean ages at sampling in the groups with either a partially or an unsuitable slide were similar at 4.9 and 4.8 days respectively.

7.3 Red blood cell morphology

There were 123 slides suitable for assessment of the red blood cell morphology. The most commonly noted red blood cell feature was the presence of nucleated red blood cells, which were seen in 68/123 (55.3%) slides. Spherocytes were seen in 52/123 (42.3%) slides; Howell-Jolly bodies were seen in 44/123 (35.8%) slides; and target cells were seen in (34.1%) slides. Basophilic stippling was uncommon, being noted in 14/123 slides (11.4%). The frequency of the different red blood cell features is shown in Figure 7-1.
Figure 7-1. The frequency of specific red blood cell features in blood films from neonates with DS.

Figure 7-2. The frequency of specific neutrophil features in blood films from neonates with DS.
7.4 Neutrophil morphology

There were 49 slides which were suitable for assessment of the neutrophil morphology. The most common finding was of hypogranular neutrophils which were noted in 46/49 (93.9%) slides. Vacuolated neutrophils were seen in 38/49 (77.6%) slides and Pelgeroid neutrophils in 26/49 (53.1%) slides. Agranular and hypersegmented neutrophils were present in 7/49 (14.3%) slides. This is shown in Figure 7-2.

As each cell was individually scored, it was also possible to calculate the percentage of cells with each feature. Hypogranularity was present in 40% neutrophils and neutrophil vacuolation in 24.3% neutrophils whereas Pelgeroid, agranular and hypersegmented neutrophils were present in fewer than 10% of cells. This is shown in Table 7-2.

Table 7-2.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Total slides reviewed</th>
<th>Slides with specific feature present number</th>
<th>Overall percentage of cells with the specific feature %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypogranular neutrophils</td>
<td>49</td>
<td>46</td>
<td>93.9</td>
</tr>
<tr>
<td>Neutrophil vacuolation</td>
<td>49</td>
<td>38</td>
<td>77.6</td>
</tr>
<tr>
<td>Pelgeroid neutrophils</td>
<td>49</td>
<td>26</td>
<td>53.1</td>
</tr>
<tr>
<td>Agranular neutrophils</td>
<td>49</td>
<td>7</td>
<td>14.3</td>
</tr>
<tr>
<td>Hypersegmented neutrophils</td>
<td>49</td>
<td>7</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Table 7-2. The frequency of neutrophil abnormalities in neonatal blood films from children with DS.
Figure 7-3. Photographs of specific morphological features in neonates with DS.

A. Megakaryocyte at the edge of a film from a neonate with TMD; B. Micro megakaryocyte at the edge of a film from a neonate without TMD; C. Megakaryocyte fragment.
D. Megakaryocyte fragment in the centre, with giant platelets and nucleated red blood cells; E. Giant platelet with blast cells and a nucleated red blood cell; F. Giant platelet with pallor, blast, nucleated red blood cell and neutrophil with poor nuclear lobation.
G and H. Hypogranular neutrophils with Pelgeroid nuclei. I. Eosinophil with Pelgeroid nucleus.
7.5 Blast cells

There were 51 slides which were suitable for a white blood cell differential count, and blasts were present in 29 (57%) of these. In the majority of cases a blast type was not ascribed. However, myeloblasts were reported in 5/29 (17%) cases with blasts and lymphoblasts in 1/29 (3%) cases. Cytoplasmic blebbing was not common, being reported in 3/29 (10%) cases only. A morphological diagnosis of TMD was made on 8/189 (4.2%) films.

During the review process it was evident that although there were slides where the morphology had degenerated so that a white blood cell differential might not be performed accurately, it was still possible to identify blasts in many of these slides. This led to a repeat review of all the slides deemed only partially suitable and of those deemed unsuitable for review to identify those where blasts were clearly present. At this second review the presence or absence of nucleated red blood cells was also ascertained. The results of this second review are given in Table 7-3.

Table 7-3. Results of second review to determine only presence of blasts and nucleated red blood cells.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Suitable for full morphology review</th>
<th>Suitable for limited morphology review</th>
<th>Unsuitable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>189</td>
<td>51</td>
<td>72</td>
<td>66</td>
</tr>
<tr>
<td>Blasts present</td>
<td>92</td>
<td>(48.7)</td>
<td>(56.9)</td>
<td>(37.9)</td>
</tr>
<tr>
<td>(number; %)</td>
<td></td>
<td>29</td>
<td>38</td>
<td>25</td>
</tr>
<tr>
<td>Nucleated red blood</td>
<td>111</td>
<td>(58.7)</td>
<td>(49)</td>
<td>(65.2)</td>
</tr>
<tr>
<td>cells present</td>
<td></td>
<td>25</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>(number; %)</td>
<td></td>
<td></td>
<td>(59.7)</td>
<td></td>
</tr>
</tbody>
</table>
7.6 Comparison of manual and automated methods for identifying blasts

As well as generating numerical parameters the automated analyser is able to identify when abnormal cells are present and generates appropriate flags, which are given as text comments on the report. The ability of the analyser to identify samples with blasts was examined. If the two flags which specifically suggested blasts were present then just over half of the samples had blasts on manual review, so that a blast flag had a sensitivity of 72% and a specificity of 43%. If three further flags which highlighted a more general white blood cell abnormality, such as the presence of immature granulocytes, were also included then the sensitivity improved to 90%, but the specificity dropped to 17%. This is shown in Table 7-4.

When the sensitivity for identifying blasts in films which were subsequently given a morphological diagnosis of TMD were considered the sensitivity with the two blast flags was 50%, and when the three abnormal white cell flags were also added in this improved to 75%.

<table>
<thead>
<tr>
<th></th>
<th>Blasts on manual review</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>absent</td>
</tr>
<tr>
<td>Automated blast flag</td>
<td></td>
</tr>
<tr>
<td>absent</td>
<td>38/164</td>
</tr>
<tr>
<td>present</td>
<td>50/164</td>
</tr>
<tr>
<td>Automated blast or abnormal white cell flag</td>
<td></td>
</tr>
<tr>
<td>absent</td>
<td>15/164</td>
</tr>
<tr>
<td>present</td>
<td>72/164</td>
</tr>
<tr>
<td></td>
<td>21/164</td>
</tr>
<tr>
<td></td>
<td>55/164</td>
</tr>
<tr>
<td></td>
<td>8/164</td>
</tr>
<tr>
<td></td>
<td>69/164</td>
</tr>
</tbody>
</table>

Table 7-4. Comparison of manual and automated methods for identifying blasts

7.7 Platelet morphology

There were 123 slides suitable for assessment of the platelet morphology. Giant platelets and/or megakaryocyte fragments were seen in 107/123 (87%) slides. The platelet colour appeared abnormal in 81 (65.9%), and in all of these cases platelets were considered pale.

Photographs of these features are provided in
Figure 7-3.
Chapter 8 Results: haematological values beyond the neonatal stage

8.1 Introduction

This chapter presents the results of the full blood count analysis for children in the CDSS beyond the neonatal period. Values are expressed as the mean (95% CI) and range (5-95% percentiles). Where results are referred to as significant, this indicates that p<0.05.

8.2 Case characteristics

These are provided in Table 8-1.

<table>
<thead>
<tr>
<th>Number</th>
<th>Cases with a sample at 0-3 months</th>
<th>Cases with a sample at 1st birthday</th>
<th>Cases with a sample at 2nd birthday</th>
<th>Cases with a sample at 3rd birthday</th>
<th>Cases with a sample at 4th birthday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: boys</td>
<td>134 (54%)</td>
<td>24 (50%)</td>
<td>7 (70%)</td>
<td>12 (86%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>girls</td>
<td>113 (46%)</td>
<td>24 (50%)</td>
<td>3 (30%)</td>
<td>2 (14%)</td>
<td>2 (40%)</td>
</tr>
</tbody>
</table>

Table 8-1. Case characteristics for children with samples in the analysis of haematological parameters beyond the neonatal stage.

8.3 Red blood cells

The striking finding for all the red blood parameters is the difference between the results at birth compared with the results at 1 year and the subsequent similarity between the results at 1, 2, 3 and 4 years. Moreover, there is no overlap between the 95% CI for the mean values of red blood cell parameters at birth compared with results at 1, 2, 3 and 4 years (p<0.05).

Considering the changes in the values between birth and 1 year, the red cell count fell from 5.4 (4.0-6.6) x10^12/l to 4.5 (3.9-5.1) x10^12/l; haemoglobin fell from 20.3 (13.8-24.8) g/dl to 13.1 (10.8-14.6) g/dl; mean cell haemoglobin fell from 37.2 (32.9-41.1) pg to 28.8 (25.2-31.5) pg; haematocrit fell from 0.62 (0.45-0.75) to 0.42 (0.33-0.53); mean cell volume fell from 114.5 (100.0-127.8) fl to 91.9 (80.6-111.0) fl; and the nucleated red blood cell count fell from 2.68 (0-14.1) x 10^9/l to 0.01 (0-0.04) x 10^9/l. Nucleated red blood cells were absent after 1 year of age. The difference between the values at birth and the subsequent values at the other age points is particularly striking when the mean cell haemoglobin is considered. Here, there is no overlap between the range at birth and the four subsequent ranges, all of which are similar to one another. These results are shown in Table 8-2, Figure 8-1 and Figure 8-2.
Table 8-2. Mean values for red blood count parameters in children with DS up to and including their 4\textsuperscript{th} birthday.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Birth\textsuperscript{a}</th>
<th>1 year\textsuperscript{b}</th>
<th>2 years\textsuperscript{b}</th>
<th>3 years\textsuperscript{b}</th>
<th>4 years\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean (95% CI)</td>
<td>N  Mean (95% CI)</td>
<td>N  Mean (95% CI)</td>
<td>N  Mean (95% CI)</td>
<td>N  Mean (95% CI)</td>
</tr>
<tr>
<td>Red cell count (x10^{12} cells/l)</td>
<td>245 5.4 (5.3, 5.5)</td>
<td>48 4.5 (4.4, 4.7)</td>
<td>10 4.5 (4.2, 4.8)</td>
<td>14 4.6 (4.4, 4.8)</td>
<td>5 4.5 (4.3, 4.7)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>245 20.3 (19.9, 20.7)</td>
<td>48 13.1 (12.5, 13.6)</td>
<td>10 13.0 (12.6, 13.4)</td>
<td>14 13.5 (12.7, 14.3)</td>
<td>5 13.4 (13.0, 13.8)</td>
</tr>
<tr>
<td>Mean cell haemoglobin (pg)</td>
<td>245 37.2 (36.9, 37.6)</td>
<td>48 28.8 (28.1, 29.4)</td>
<td>10 29.0 (27.9, 30.1)</td>
<td>14 29.2 (28.5, 30.0)</td>
<td>5 30.0 (28.9, 31.0)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>242 0.62 (0.62, 0.63)</td>
<td>48 0.42 (0.40, 0.44)</td>
<td>10 0.43 (0.41, 0.45)</td>
<td>14 0.44 (0.40, 0.48)</td>
<td>5 0.40 (0.39, 0.41)</td>
</tr>
<tr>
<td>Mean cell volume (fl)</td>
<td>244 114.5 (113.4, 115.6)</td>
<td>48 91.9 (89.3, 94.4)</td>
<td>10 96.0 (90.4, 100.5)</td>
<td>14 95.4 (90.4, 100.5)</td>
<td>5 89.4 (85.1, 93.6)</td>
</tr>
<tr>
<td>Nucleated RBC (x10^{9} cells/l)</td>
<td>208 2.68 (1.7, 3.66)</td>
<td>47 0.01 (0, 0.04)</td>
<td>9 0</td>
<td>13 0</td>
<td>4 0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} birth - 3 months  
\textsuperscript{b} birthday ± 3 months
Table 8-3. Range of values for red blood count parameters in children with DS up to and including their 4th birthday.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Birth</th>
<th>1 year</th>
<th>2 years</th>
<th>3 years</th>
<th>4 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Range (95% percentile)</td>
<td>N</td>
<td>Range (95% percentile)</td>
<td>N</td>
</tr>
<tr>
<td>Red cell count (x10^{12} cells/l)</td>
<td>245</td>
<td>4.0-6.6</td>
<td>48</td>
<td>3.9-5.1</td>
<td>10</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>245</td>
<td>13.8-24.8</td>
<td>48</td>
<td>10.8-14.6</td>
<td>10</td>
</tr>
<tr>
<td>Mean cell haemoglobin (pg)</td>
<td>245</td>
<td>32.9-41.1</td>
<td>48</td>
<td>25.2-31.5</td>
<td>10</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>242</td>
<td>0.45-0.75</td>
<td>48</td>
<td>0.33-0.53</td>
<td>10</td>
</tr>
<tr>
<td>Mean cell volume (fl)</td>
<td>244</td>
<td>100.0-127.8</td>
<td>48</td>
<td>80.6-111.0</td>
<td>10</td>
</tr>
<tr>
<td>Nucleated RBC (x10^9 cells/l)</td>
<td>208</td>
<td>0-14.1</td>
<td>47</td>
<td>0-0.04</td>
<td>9</td>
</tr>
</tbody>
</table>

a. birth - 3 months
b. birthday ± 3 months
Figure 8-1. Mean values for haemoglobin, haematocrit and red cell count for children with DS from birth up to and including their 4th birthday.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval.
Figure 8-2. Mean values for mean cell haemoglobin, mean cell volume and nucleated red cell count for children with DS from birth up to and including their 4th birthday.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval.
8.4 White blood cell parameters

Again, there appears to be a marked difference in the total white cell count, and in all the white blood cell subtypes, between birth and the results at 1 year and a subsequent similarity between the results at 1, 2, 3 and 4 years. Again, there is no overlap between the 95% CI for mean values of white blood cell parameters at birth compared with results at 1, 2, 3 and 4 years indicating that this is a significant (p<0.05). The exception to this is the eosinophil count where there is the same trend, but where there is overlap between the 95% CI.

Considering the changes in the values between birth and 1 year, the total white blood cell count fell from 11.7 (5.9-23.8) x 10^9/l to 6.8 (4.2-9.9) x 10^9/l; the neutrophil count fell from 6.6 (1.9-15.8) x 10^9/l to 3.2 (1.5-7.4) x 10^9/l; the lymphocyte count fell from 3.7 (1.5-7.4) x 10^9/l to 2.8 (1.5-4.5) x 10^9/l; the monocyte count fell from 1.0 (0.3-2.0) x 10^9/l to 0.6 (0.3-1.3) x 10^9/l; the basophil count fell from 0.17 (0-0.52) x 10^9/l to 0.09 (0.02-0.21) x 10^9/l; and the eosinophil count fell from 0.17 (0-0.47) x 10^9/l to 0.14 (0-0.30) x 10^9/l.

The ranges tend to become narrower from the 1st birthday onwards. Moreover, this occurs because the upper limit decreases while the lower limit remains relatively constant. These results are shown in Table 8-4, Table 8-5, Figure 8-2, Figure 8-3 and Figure 8-4.
Table 8-4. Mean values for white blood count parameters in children with DS up to and including their 4th birthday.

<table>
<thead>
<tr>
<th></th>
<th>Birth&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1 year&lt;sup&gt;b&lt;/sup&gt;</th>
<th>2 years&lt;sup&gt;b&lt;/sup&gt;</th>
<th>3 years&lt;sup&gt;b&lt;/sup&gt;</th>
<th>4 years&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (95% CI)</td>
<td>N</td>
<td>Mean (95% CI)</td>
<td>N</td>
</tr>
<tr>
<td>White cell count (x10&lt;sup&gt;9&lt;/sup&gt; cells/l)</td>
<td>130</td>
<td>11.7 (10.5, 12.9)</td>
<td>45</td>
<td>6.8 (6.2, 7.4)</td>
<td>10</td>
</tr>
<tr>
<td>Neutrophils (x10&lt;sup&gt;9&lt;/sup&gt; cells/l)</td>
<td>130</td>
<td>6.6 (5.7, 7.5)</td>
<td>45</td>
<td>3.2 (2.7, 3.7)</td>
<td>10</td>
</tr>
<tr>
<td>Lymphocytes (x10&lt;sup&gt;9&lt;/sup&gt; cells/l)</td>
<td>130</td>
<td>3.7 (3.3, 4.1)</td>
<td>45</td>
<td>2.8 (2.5, 3.1)</td>
<td>10</td>
</tr>
<tr>
<td>Basophils (x10&lt;sup&gt;9&lt;/sup&gt; cells/l)</td>
<td>130</td>
<td>0.17 (0.14, 0.21)</td>
<td>45</td>
<td>0.09 (0.07, 0.11)</td>
<td>10</td>
</tr>
<tr>
<td>Eosinophils (x10&lt;sup&gt;9&lt;/sup&gt; cells/l)</td>
<td>130</td>
<td>0.17 (0.14, 0.19)</td>
<td>45</td>
<td>0.14 (0.09, 0.19)</td>
<td>10</td>
</tr>
<tr>
<td>Monocytes (x10&lt;sup&gt;9&lt;/sup&gt; cells/l)</td>
<td>130</td>
<td>1.0 (0.9, 1.1)</td>
<td>45</td>
<td>0.6 (0.5, 0.6)</td>
<td>10</td>
</tr>
</tbody>
</table>

<sup>a</sup> birth - 3 months  
<sup>b</sup> birthday ± 3 months
Table 8-5. Range of values for white blood count parameters in children with DS up to and including their 4th birthday.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Birth&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1 year&lt;sup&gt;b&lt;/sup&gt;</th>
<th>2 years&lt;sup&gt;a&lt;/sup&gt;</th>
<th>3 years&lt;sup&gt;b&lt;/sup&gt;</th>
<th>4 years&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N, Range (95% percentile)</td>
<td>N, Range (95% percentile)</td>
<td>N, Range (95% percentile)</td>
<td>N, Range (95% percentile)</td>
<td>N, Range (95% percentile)</td>
</tr>
<tr>
<td>White cell count (x10^9 cells/l)</td>
<td>130 5.9-23.8</td>
<td>45 4.2-9.9</td>
<td>10 3.4-10.7</td>
<td>13 4.7-7.4</td>
<td>5 4.6-10.0</td>
</tr>
<tr>
<td>Neutrophils (x10^9 cells/l)</td>
<td>130 1.9-15.8</td>
<td>45 1.5-7.4</td>
<td>10 1.1-6</td>
<td>13 2.3-4.6</td>
<td>5 1.9-6.6</td>
</tr>
<tr>
<td>Lymphocytes (x10^9 cells/l)</td>
<td>130 1.5-7.4</td>
<td>45 1.5-4.5</td>
<td>10 1.3-3.0</td>
<td>13 1.3-2.7</td>
<td>5 1.7-2.6</td>
</tr>
<tr>
<td>Basophils (x10^9 cells/l)</td>
<td>130 0.0-0.52</td>
<td>45 0.02-0.21</td>
<td>10 0.04-0.19</td>
<td>13 0.02-0.2</td>
<td>5 0.05-0.13</td>
</tr>
<tr>
<td>Eosinophils (x10^9 cells/l)</td>
<td>130 0.0-0.47</td>
<td>45 0.01-0.3</td>
<td>10 0.04-0.34</td>
<td>13 0.03-0.33</td>
<td>5 0.07-0.34</td>
</tr>
<tr>
<td>Monocytes (x10^9 cells/l)</td>
<td>130 0.3-2.0</td>
<td>45 0.3-1.3</td>
<td>10 0.1-1.3</td>
<td>13 0.2-1.1</td>
<td>5 0.3-0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> birth - 3 months
<sup>b</sup> birthday ± 3 months
Figure 8-3. Mean values for white cell, neutrophil and lymphocyte count for children with DS from birth up to and including their 4th birthday.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval.
Figure 8-4. Mean values for monocyte, basophil and eosinophil count for children with DS from birth up to and including their 4th birthday.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval.
8.5 Platelets

Again, there appears to be a marked difference in the platelet count and the mean platelet volume between birth and the results at 1 year and a subsequent similarity between the results at 1, 2, 3 and 4 years. Again, there is no overlap between the 95% CI for mean values for the platelet parameters at birth compared with results at 1, 2, 3 and 4 years indicating that this is a significant difference.

The mean platelet count increases between birth and 1 year rising from $160 (38-317) \times 10^9/l$ to $308 (152-483) \times 10^9/l$. The mean value for mean platelet volume decreases between birth and 1 year falling from $12.1 (10.5-13.9) \text{ fl}$ to $10.3 (8.7-11.9) \text{ fl}$.

It can be seen that both the upper and lower limits increase for the platelet count from birth to the 1st birthday and onwards. This increase is most marked for the lower value which increases from $35 \times 10^9/l$ at birth to $152 \times 10^9/l$ at 1 year. In contrast, the upper and lower limits for the mean platelet volume both fall from birth to the 1st birthday and onwards. These results are shown in Table 8-6, Table 8-7 and Figure 8-5.
Table 8-6. Mean values for platelet parameters in children with DS up to and including their 4th birthday.

<table>
<thead>
<tr>
<th></th>
<th>Birth&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1 year&lt;sup&gt;b&lt;/sup&gt;</th>
<th>2 years&lt;sup&gt;b&lt;/sup&gt;</th>
<th>3 years&lt;sup&gt;b&lt;/sup&gt;</th>
<th>4 years&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (95% CI)</td>
<td>N</td>
<td>Mean (95% CI)</td>
<td>N</td>
</tr>
<tr>
<td>Platelet count (x10⁹ cells/l)</td>
<td>238</td>
<td>160 (150, 170)</td>
<td>46</td>
<td>308 (279, 338)</td>
<td>10</td>
</tr>
<tr>
<td>Mean platelet volume (fl)</td>
<td>80</td>
<td>12.1 (11.9, 12.3)</td>
<td>39</td>
<td>10.3 (10.0, 10.6)</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> birth - 3 months  
<sup>b</sup> birthday + 3 months
<table>
<thead>
<tr>
<th></th>
<th>Birth(^a)</th>
<th>1 year(^b)</th>
<th>2 years(^b)</th>
<th>3 years(^b)</th>
<th>4 years(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet count (x10^9 cells/l)</strong></td>
<td>238 38-317</td>
<td>46 152-483</td>
<td>10 160-387</td>
<td>14 169-389</td>
<td>5 195-363</td>
</tr>
<tr>
<td><strong>Mean platelet volume (fl)</strong></td>
<td>80 10.5-13.9</td>
<td>39 8.7-11.9</td>
<td>8 8.8-11.9</td>
<td>10 8.5-12.9</td>
<td>5 9.6-11</td>
</tr>
</tbody>
</table>

\(^a\) birth - 3 months  
\(^b\) birthday + 3 months
Figure 8-5. Mean values for platelet count and mean platelet volume for children with DS from birth up to and including their 4th birthday.

Black squares indicate mean values (+/- standard error) of the parameter at each day interval.
Chapter 9 Discussion

The central hypothesis underpinning this research is that if characterised in as near a population based cohort as possible then the haematological profile of neonates with DS will prove to be distinct. Accordingly, the primary aims of this work were:

1. To describe the neonatal haematological ranges for children with DS with reference to the standard haematological parameters typically measured as part of full blood count analysis;

2. To examine factors that may be associated with variations in the neonatal haematological profile of children with DS;

3. To describe the features of neonatal blood cell morphology in children with DS;

4. To describe the haematological parameters in children with DS beyond the neonatal stage.

Each of these aims will be discussed in turn in this chapter and their outcome reviewed.

9.1 Case characteristics

This is the largest data set to date of full blood count parameters in neonates with DS, and this remains the case even when the set is restricted to those cases with a full blood count sample taken in the first week of life. Moreover, it is the only prospective analysis considering haematological parameters throughout the neonatal period.

In both the neonatal and in the first week of life analysis there is a male preponderance with 53% of cases being male. A male excess in DS births has been consistently noted elsewhere (Kovaleva, 2002; Bloemers et al, 2007).

Just over a third (37%) births were preterm, this fell slightly to 32% for the first week of life analysis. There are various possible reasons for the difference between the groups: families with a preterm newborn baby might be less likely to be approached about the study in the first week of life as the babies might be sicker; it might be perceived to be less urgent as they were likely to be in hospital for longer; and even if they were recruited within the first week of life venepuncture might be put off until they were larger. In the CDSS the mean gestational age was lower than the general population – as noted by other groups. The birth cohort reported by the Dutch Paediatric Surveillance Unit (DPSU) reported a mean gestational age
of 38 weeks, compared with 39.1 weeks for their control group (Weijerman et al, 2008). The Intermountain Healthcare Hospitals study of haematological parameters within the first week in neonates with DS reported a mean gestational age of 37.4 +/- 1.9 weeks (Henry et al, 2007).

In the CDSS just under a quarter of babies (24%) had a birth weight of <2500 grams. This falls to a fifth (20%) for the first week of life analysis which may reflect the reduction in preterm births in that set. Birth weight was taken from the initial hospital referral form, from the family questionnaire and from the obstetric note abstraction. Not all cases have been abstracted so far, and not all families completed a family questionnaire, but all had a hospital referring form. Despite this the weight was missing for just under a sixth of cases (14% for the neonatal analysis; 15% for the first week of life analysis). The DPSU reported a mean birth weight of 3119 grams in boys compared with 3525 grams for controls, and 2901 grams in girls compared with 3389 grams for controls (Weijerman et al, 2008). The Intermountain Healthcare Hospitals study reported a mean birth weight of 2894 +/- 618 grams (Henry et al, 2007).

The male excess observed in the neonatal haematology analysis was only observed in cases with slides suitable for full morphological analysis. However, the earlier gestational age and lower birth weight were similar for cases in the neonatal haematology and neonatal morphology analyses.

9.2 Haematological parameters in DS neonates

The findings of the CDSS neonatal analysis:

1. Support the hypothesis that neonates with DS have a distinct haematological profile;
2. Have enabled the characterisation of haematological parameters throughout the neonatal period in a comprehensive manner with the production of charts giving mean and range values for the CDSS population - this has not been done before;
3. Have demonstrated the association of the neonatal haematological parameters with birth weight, gestational age, sampling to processing interval and postnatal age, providing novel insight into the role of these variables.

The findings are summarised in Table 9-1, and are then discussed below.
Table 9-1. Summary of the findings of the neonatal analysis of haematological parameters for the CDSS.

<table>
<thead>
<tr>
<th></th>
<th>Change compared with wider neonatal population</th>
<th>Change with increasing birth weight</th>
<th>Change with increasing gestational age</th>
<th>Change with increasing postnatal age</th>
<th>Cut-off for stability (days)</th>
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<td>Haematocrit</td>
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<td>Red blood cell count</td>
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<td>Nucleated red blood cell count</td>
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<td>Monocytes</td>
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<td><strong>PLATELET PARAMETERS</strong></td>
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<td>Mean platelet volume</td>
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</tbody>
</table>

Arrows in blue indicate a difference from the wider neonatal population.
9.2.1 First week of life analysis: red blood cells

Samples taken within the first week of life were considered first as many of the comparable reports are limited to this period. This is also the period in which any effects of gestational age or birth weight are likely to be most pronounced and so the regression analysis was performed on this data set.

Haemoglobin

The haemoglobin and haematocrit both appear higher than the reference values for neonates. Here the haemoglobin was 21.1 (16.8-25.3) g/dl. The mean for the general population of neonates age 3 days studied by the Intermountain group was 16.6 g/dl which is outside the CDSS range, while the mean for the CDSS group is higher than the upper limit of the range for the Intermountain group which was 20.9 g/dl (95% percentile value) (Jopling et al, 2009). Similarly, the mean reported for neonates at 1 week is 17.5 g/dl, which is right at the bottom of the CDSS range (Orkin & Nathan 2009).

There are five reports which consider the haemoglobin in DS neonates (Behrman, Sigler and Patchefsky, 1966; Thüring and Tönz, 1979; Lappalainen and Kouvalainen, 1972; Kivivuori, Rajantie and Siimes, 1996; Henry et al, 2007). However, one of these consists of 3 case reports only (Behrman, Sigler and Patchefsky, 1966); one only considers the effect of haemoglobin on the platelet count (Thüring and Tönz, 1979); one does not give either figures or graphical illustration (Lappalainen and Kouvalainen, 1972); and in the small prospective study the data is presented graphically only over a 12 month period so that the numbers cannot be accurately extrapolated (Kivivuori, Rajantie and Siimes, 1996). The largest and most recent paper plots the individual data points against what it considers a reference range of 13-22 g/dl (Henry et al, 2007). It can be seen on this graph that there are consistently more data points near and above the upper limit than the lower one, especially in the first 72 hours of life. Moreover, in the text of the paper the authors state that one third of haemoglobin results were above 22 g/dl, and 14 % of results above 23 g/dl. This is consistent with the findings here that the haemoglobin in DS neonates appears increased.

Haematocrit

The haematocrit was 0.6 (0.51-0.76) compared with 0.49 (0.36-0.62) (mean; 95% percentile values) from the Intermountain group for neonates age 3 days (Jopling et al, 2009) so that the CDSS mean haematocrit is right at the upper limit of the range for the wider population while the mean for the wider population is below the lower limit of the range for the CDSS.
population. The mean reported for neonates at 1 week is 0.54, which, although higher than that reported by the Intermountain group, is still lower than the CDSS mean (Orkin and Nathan, 2009).

Although polycythaemia was the first red blood cell abnormality to be described in children with DS (Weinberger and Oleinick, 1970), most of the papers do not give either mean values or reference ranges. In the Intermountain paper on DS neonates the individual data points are plotted against an estimated reference range for haematocrit of 0.4-0.6 (Henry et al, 2007). Again, the results tended to be near and above the upper limit, with the haematocrit being greater than 0.65 and 0.7 in one third and 14 % cases respectively. This is consistent with the findings here that the haematocrit in DS neonates appears increased.

When the haemoglobin and haematocrit findings are considered together 95 (54%) of the 176 cases were polycythaemic (total haemoglobin was greater than 22 g/dl or the haematocrit was greater than 0.65) in the first week of life. The incidence of polycythaemia for all term neonates has been consistently reported as being between 2-5% (Wirth et al. 1979; Ramamurthy & Brans 1981; Reisner et al. 1983). This work suggests that there is a ten to twenty fold increase in polycythaemia in DS. The significance of neonatal polycythaemia is yet to be fully elucidated – whether or not this occurs in the context of DS. It is known that cerebral blood flow is decreased in neonates with polycythaemia (Rosenkrantz and Oh, 1982), and a number of studies have reported a variety of symptoms that have been observed in polycythaemic neonates (Gross, Hathaway and McGaughey, 1973; Ramamurthy and Brans, 1981; Black et al, 1982). However, the frequency of these symptoms varies considerably between reports – for example, cyanosis is reported to occur in 89% and tremor or jitters in 67% in one study (Gross, Hathaway and McGaughey, 1973) compared with a frequency of 17% and 13% respectively in another study (Ramamurthy and Brans, 1981). The numbers in these studies are small, with even the largest only considering 111 infants (Black et al, 1982). Moreover, the nature of the association is unclear and many of the symptoms described appear likely to be a consequence of the original insult that led to polycythaemia rather than a consequence of it.

It is possible to lower the haematocrit with partial exchange transfusion (PET), but there is no evidence to support this in an asymptomatic infant. Its benefit in symptomatic infants is unclear. PET has been associated with an increase in necrotising enterocolitis (Black et al, 1985a) and does not appear to be associated with a more rapid improvement in neurological symptoms (Goldberg et al, 1982). A recent Cochrane review of the use of partial exchange
transfusion (PET) for neonatal polycythaemia to prevent neuro-disability concluded that there was no proven clinically significant short or long-term benefits of PET in polycythaemic newborn infants who were clinically well or who had only minor symptoms related to hyperviscosity (Ozek, Soll and Schimmel, 2010). It also found an apparent increase in necrotizing enterocolitis in neonates treated with PET. However, follow-up data were imprecise so that the true risks and benefits of PET are still unclear. There have been a number of longitudinal studies of polycythaemic infants. These agree that infants with polycythaemia are at increased risk of subsequent neurological sequelae and this risk does not appear to be modified by PET (Black et al, 1982; Black et al, 1985b; Delaney-Black et al, 1989; Bada et al, 1992). They conclude that there is an initial hypoxic event – which may be an adverse intrauterine environment, fetal hypoxia or asphyxia – which results in an adaptive, erythropoietin driven, polycythaemia coincident with neurological damage.

Children with DS have typically been excluded from the above studies. It is not yet known whether there is a similar association with neonatal polycythaemia and subsequent neurological sequelae, or whether there is any role for PET. A study of erythropoietin levels in the umbilical cord blood of 18 neonates with DS and 36 control infants did demonstrate a significantly increased erythropoietin level in the DS group (Widness et al, 1994). Erythropoietin does not cross the placenta and so is assumed to be of fetal origin (Jacobson, Marks and Gaston, 1959). Increased levels of fetal erythropoietin have previously been shown to be associated with intrauterine hypoxaemia and intrauterine growth retardation (Snijders et al, 1993). Erythropoietin is a critical stimulator of erythropoiesis. Hypoxia results in increased transcription of the erythropoietin gene, and increased erythropoietin levels, which in turn increases erythropoiesis (Wang and Semenza, 1995).

Red blood cell count

The red blood cell count is an often overlooked parameter. The red blood cell count here of 5.6 x10^{12} cells/l is only slightly higher than a mean of 5.1 x10^{12} cells/l reported for neonates at 1 week in the wider population, and the mean for the wider population is well within the CDSS range of 4.3-6.6 x10^{12} cells/l suggesting that there is not a marked difference between the two groups (Orkin and Nathan, 2009). It has not been reported for DS neonates before.

Mean cell haemoglobin

Given that the haemoglobin is notably higher but the red blood cell count only slightly higher than the general population values it is unsurprising that the mean cell haemoglobin also appears to be higher in the CDSS than the general population. The mean cell haemoglobin
was 37.7 (33.8-41.3) pg compared with 36.6 (32.9-40.4) pg (weighted mean; 95% percentile values) for neonates aged 1 day (Christensen et al, 2008) or mean 34 pg for neonates at 1 week (Orkin and Nathan, 2009). Again, there are no comparable reports of the mean cell haemoglobin in DS neonates. It is mentioned briefly in the prospective study of 25 neonates with DS (Kivivuori, Rajantie and Siimes, 1996). Although no numbers are given, the authors note that the values are close to the upper range of reference values.

Mean cell volume

It has been recognised for a long time that the red blood cells of the fetus and neonates are larger than in later childhood or adults. The red blood cells appear to be larger still in DS neonates. The mean cell volume was 115.5 (102.1-128.5) fl compared with 107.8 (98-117.6) fl (weighted mean; 95% percentile values) for neonates aged 1 day (Christensen et al, 2008) or mean 107 fl for neonates at 1 week (Orkin and Nathan, 2009) or mean 110 fl at birth taken from the paper previously considered as standard reference (Matoth, Zaizov and Varsano, 1971). An increased mean cell volume is seen in premature compared with term neonates in the general population reflecting splenic immaturity (Diagne et al, 1995). It is thought that the mean cell volume decreases with increasing gestational age as the spleen becomes more mature and is able to perform more effective remodelling of the red cell membrane (Orkin and Nathan, 2009). The mean cell volume values for the CDSS population are comparable to those of neonates of 26-28 weeks gestation (Christensen et al, 2008) or to fetuses sampled at 29-30 weeks gestation (Forestier et al, 1991). Macrocytosis has been recognised in adults and children with DS, but has only been reported in DS neonates more recently. The small prospective study of DS neonates (Kivivuori, Rajantie and Siimes, 1996) give values for the mean red cell volume in the first week with values of 110 (79-124) fl (mean; 95% percentile values). This is similar to the larger retrospective study which quotes 110 ± 7 fl (mean; 2 standard deviations) although the graph indicates that these values are higher in the first 48 hours (Henry et al, 2007). Our values were slightly higher than this which may reflect timing as most of the CDSS samples were taken within the first few days of life when the mean cell volume is highest.

Nucleated red blood cells

Nucleated red blood cells are expected to disappear from the peripheral circulation by the 4th day of life (Bain, 2002). The mean nucleated red blood cell count of 3.8 (0-20.3) x10^9 cells/l for the first week of life in this group would appear to be higher than the value of 2.3 (0-6.4) x10^9 cells/l (mean; 25-75th percentile values) reported for the general population (Perrone et
An increased or persisting nucleated red blood cell count has been recognised as an important marker of perinatal brain damage since the 1990s (Green, Hendon and Mimouni, 1995; Buonocore et al, 1999). Subsequent reports have consistently found an association between nucleated red blood cell count and adverse perinatal outcomes. A recent study which looked at over 35 000 results found a four-fold increase in intraventricular haemorrhage and in retinopathy of prematurity in cases where the nucleated red blood cell count was over 95% of the reference ranges determined for that population (Christensen et al, 2011). The authors conclude that an increase in nucleated red blood cells is a marker of perinatal hypoxia occurring as a result of a hypoxia-driven increase in erythropoietin. In support of this they note that an increase in erythropoietin levels has been demonstrated in many conditions associated with perinatal hypoxia. Importantly, an increase in erythropoietin has also been demonstrated in the cord blood of neonates with DS (Widness et al, 1994). They also note that hepatic haematopoiesis is still making a significant contribution to haematopoiesis at birth, especially in preterm births, and suggest that nucleated red blood cells may lose their nucleus less readily on leaving the liver than the bone marrow. This latter idea is hypothetical.

Taken together this work suggests that the red blood cells of neonates with DS within the first week of life are similar in number, but larger and more haemoglobin rich with a consequent increase in haematocrit than in neonates without DS. There is a concurrent increase in nucleated red blood cells which may be a marker of perinatal hypoxia.

9.2.2 First week of life analysis: white blood cell parameters

Total white blood cell count

The total white cell count in the CDSS group appears very similar to values obtained for the wider population with a white cell count of 12.9 (6.1-24.2) x10⁹ cells/l for the CDSS compared with a reference range of 12.2 (5.0-21.0) x10⁹ cells/l (mean; 95% CI) for the first week of life in the general population (Orkin and Nathan, 2009).
Differential white blood cell count

However, when the differential count is examined the story becomes more interesting as the neutrophil component appears increased with a relative lymphopenia in the CDSS. The CDSS neutrophil count was 8 (1.9-16) x10⁹ cells/l compared with 5.5 (1.5-10) x10⁹ cells/l (mean; 95% CI) for the first week of life (Orkin and Nathan, 2009), or 5.4 (1.3-13) x10⁹ cells/l (weighted mean; 95% percentile values) for neonates aged 3 days (Schmutz et al, 2008). This finding is consistent with the large retrospective study of neonates with DS which also found a relative neutrophilia, noting that 80% had a neutrophilia when compared with the Manroe ranges (Manroe et al, 1979) and also remarking that only 1/158 cases had a neutrophil count < 1.0 x10⁹ cells/l (Henry et al, 2007).

Determining the neutrophil count in DS neonates is important clinically as neonatal units may use the white cell count, or more specifically the neutrophil count, in algorithms to determine the likelihood of sepsis (Verani, McGee and Schrag, 2010). However, although the neutrophil count appears to be similar, or even higher, in DS compared with the general population, it is not wise to infer that both populations will respond similarly to infection. Consequently, algorithms for neonatal sepsis which incorporate the neutrophil count need specific validation in neonates with DS.

In contrast, the lymphocyte count in the CDSS neonates appears slightly lower than in the general population at 3.6 (1.5-7.5) x10⁹ cells/l compared with 5.0 (2.0-17.0) x10⁹ cells/l (mean; 95% CI) for the first week of life (Orkin and Nathan, 2009). Although the actual lymphocyte count has not been reported before in DS neonates, it was referred to as being lower in a study of lymphocyte subpopulations in children with DS (de Hingh et al, 2005).

The monocyte count in the CDSS neonates appears similar to that of the general population at 1.0 (0.3-2.0) x10⁹ cells/l compared with mean 1.1 x10⁹ cells/l for the first week of life (Orkin and Nathan, 2009), or 1.12 (0.21-2.55) (mean; 95% CI) at 3 days (Christensen et al, 2010). The eosinophil count also appears similar, or possibly lower, at 0.2 (0-0.4) x10⁹ cells/l compared with mean 0.5 x10⁹ cells/l for the first week of life (Orkin and Nathan, 2009), or 0.47 (0.1-1.2) (mean; 95% CI) at 3 days (Christensen et al, 2010). It is not possible to compare the basophil count as there are no reference values for the general population.

Monocyte, eosinophil and basophil values have not been reported before in neonates with DS.
9.2.3 First week of life analysis: platelets

The results here indicate that the platelets of neonates with DS are reduced in number and increased in size. This relationship is important: historically, many discussions of platelets have focussed only on the platelet number. However, it is now apparent that it is platelet mass, rather than simply platelet number, which affects platelet function (Thompson and Jakubowski, 1988). An association between thrombocytopenia and increasing mean platelet volume has been demonstrated in the neonatal setting in the general population (Kipper and Sieger, 1982).

The mean platelet count in the CDSS was low at 142 (35-255) x10⁹ cells/l compared with the values reported for the general population of 271 (134-415) x10⁹ cells/l (weighted mean; 95% percentile values) by the Intermountain group for neonates at 3 days – the mean for the CDSS is right at the bottom of the range for the wider population, while the mean for the wider population is actually above the upper limit of the CDSS range. Similarly, the reported mean for neonates at 1 week is at the upper end of the CDSS range at 248 x10⁹ cells/l (Orkin and Nathan, 2009).

Thrombocytopenia was one of the earliest haematological abnormalities to be reported in DS (Behrman, Sigler and Patchefsky, 1966), and has been consistently reported in DS neonates since. However, only a few studies actually report platelet values. A study of 70 neonates in the first week of life found a platelet count of 91 (45-175) x10⁹ cells/l (median; 90% percentiles) (Thüring and Tönz, 1979); while a sample of 25 neonates with samples taken at 48 hours found values of 148 (41- 408) x10⁹ cells/l (median, range) (Hord, Gay and Whitlock, 1995). The large retrospective study of DS neonates reports a range of 20- 490 x10⁹ cells/l and states that 66% had a platelet count <150 x10⁹ cells/l including 33% with a platelet count < 100 x10⁹ cells/l and 6% with a platelet count < 50 x10⁹ cells/l (Henry et al, 2007).

The incidence of neonatal thrombocytopenia in the general population is reported to be 2-8% (Burrows and Kelton, 1990; Sainio et al, 2000) suggesting a seven to thirty fold increase in neonates with DS. Thrombocytopenia in DS is early onset –present at birth - and tends to resolve. The most common cause of early onset thrombocytopenia (defined as being present within 72 hours of birth) is chronic fetal hypoxia (Watts and Roberts, 1999). The natural history of this is spontaneous resolution and there are rarely clinical sequelae. The thrombocytopenia of DS appears to follow this pattern, and the association with chronic fetal hypoxia would be consistent with the red blood cell findings already discussed. Correlation
with clinical features, both contemporaneous and subsequent behaviour, will be helpful and will be part of future work.

In contrast the mean platelet volume here appeared higher than that of the general population. The CDSS values were 12.1 (10.4-13.9) fl compared with 8.4 (6.9-10.7) fl (weighted mean; 95% percentile values) for neonates at 3 days. The mean value for the CDSS is above the upper limit of the range for the wider population, and there is very little overlap at all between the ranges. The values here are consistent with the large retrospective analysis which reported mean platelet volume at 9.15 ± 1.3 fl (mean ± SD) with 24% values being >10 fl (Henry et al, 2007).

Larger platelets are typically more active in terms of their haemostatic function (Thompson et al, 1983; Thompson and Jakubowski, 1988), and this may explain why bleeding problems have not been described in DS neonates despite their generally low platelet counts. Whereas reticulocytes are younger and larger red blood cells which become small with increasing maturity large platelets do not appear to be immature platelets. Instead, their increased size appears to be a feature of bone marrow production factors and they remain large until the end of their life. Interestingly, these papers suggest that they have an increased life span compared with their smaller counterparts.

9.2.4 Association between birth weight and red blood cell parameters

There are no previous reports which have considered the association between birth weight and haematological parameters in DS neonates.

There have only been a few reports of the relationship between birth weight and red blood cell parameters in neonates. Changes appear to be mild and to resolve rapidly (Ozyürek et al, 2006). The lack of an association here would seem to be consistent with the wider neonatal population.

9.2.5 Association between birth weight and white blood cell parameters

There is no evidence that changes in birth weight are associated with changes in white blood cell parameters. The lack of an association here would therefore seem to be consistent with the wider neonatal population.
9.2.6 Association between birth weight and platelet parameters

The platelet count has been consistently shown to rise with increasing birth weight in the general population, with several studies comparing SGA with AGA babies (McIntosh, Kempson and Tyler, 1988; Ozyürek et al, 2006) and one comparing babies >2000 grams with those <2000 grams (Arad et al, 1986). This finding was replicated here in the neonatal CDSS population.

9.2.7 Association between gestational age and red blood cell parameters

There are no previous reports which have considered the association between gestational age and haematological parameters in DS neonates.

The association between increasing gestational age and an increase in haemoglobin, haematocrit and red cell count is consistent with that described in the general population – both in neonates (Jopling et al, 2009), and in fetuses sampled in utero (Forestier et al, 1986; Forestier et al, 1991).

9.2.8 Association between gestational age and white blood cell parameters

Although some studies have reported a lower neutrophil count in babies born preterm in the general population this effect has not been consistently reported (Lloyd and Oto, 1982; Mouzinho et al, 1992; Mouzinho et al, 1994). No significant association was found between gestational age and white blood cell parameters in the CDSS analysis.

9.2.9 Association between gestational age and platelet parameters

The finding that the platelet count fell with increasing gestational age is significant and the effect became larger when the set was restricted to those processed within 2 days, with a fall in platelet count of 30.9 (40.1, 21.8) x10^9 cells/l for each added week of gestational age. However, this is strikingly different to the relationship in the general population where studies have shown a stable platelet count with increasing gestational age in neonates (Sell and Corrigan, 1973) and in fetuses sampled in utero (Forestier et al, 1986; Forestier et al, 1991), while the largest dataset reported a small increase in the platelet count from 22 weeks gestation (Wiedmeier et al, 2009).

9.2.10 Sampling to processing interval

When the CDSS was set up it was not routine practice for children with DS to have a full blood count checked if they looked clinically well. This meant that a new sample needed to
be taken for the study in many cases as there was no local record of the full blood count results. Ideally a sample is processed in a local laboratory on the day that it is taken to minimise any artefacts introduced by a delay in processing. However, processing does not always occur on the day a sample is taken – for example samples taken in the community may be subject to transport delays. The CDSS was set up in over 60 hospitals and it was not logistically possible for the samples to be processed in each local laboratory before being sent to York for storage. It was therefore set up so that samples would be posted directly to the central haematology laboratory used by the CDSS. In order to try to minimise delays prepaid addressed padded bags were provided along with instructions for these to go directly in the post, rather than, for example, being first logged in to the local laboratory.

It was evident that this pathway would incur a delay in processing for all but those with samples taken in the same hospital as the analyser. One of the important aspects of this analysis was therefore to examine the stability of the haematological parameters with an increasing sampling to processing interval.

9.2.11 Stability of red blood cell parameters

The increase in the haematocrit and the mean cell volume with increasing sampling to processing interval is consistent with results from performance evaluation studies of the Sysmex XE-2100 which examined stability over a 68 hour period (Walters and Garrity, 2000). This found no significant variation in the haemoglobin or red blood cell count, but the haematocrit and mean cell volume were affected.

There was no evidence of a change in the stability of the nucleated red blood cell results with time. There were a number of samples for which a nucleated red blood cell count was not reported. However, the samples processed at the start of the study were analysed on an Advia 2120, which was not set to report the nucleated red blood cell count. The Sysmex XE-2100 was set to report the nucleated red blood cell count, but would not do so if the results indicated that a valid result would not be obtained. Most of the samples with a missing nucleated red blood cell count had been processed on the Advia 2120 and the random pattern of interval length in those samples was therefore to be expected as it reflected on the analyser and not the sample characteristics.
9.2.12 Stability of white blood cell parameters

Although the white blood cell count was reported on all samples, including those with a 7 day sampling to processing interval, the number with an available white blood cell differential count fell off rapidly with increasing interval time. The stability of the differential count is known to be subject to change with age, and the performance evaluation studies of the Sysmex XE-2100 reported that the differential count was stable to ~36 hours if refrigerated and to ~24 hours at room temperature (Walters and Garrity, 2000). In our set, which was a much larger sample than that used in the performance evaluation, similar results were obtained. Interestingly, the lymphocyte, eosinophil and basophil counts remained stable throughout the period examined although in practice this was 3 days for most results as the machine rarely yielded a differential count beyond this time.

9.2.13 Stability of the platelet parameters

The stability of the platelet count with increasing sampling to processing interval is consistent with the findings from the performance evaluation study (Walters and Garrity, 2000). The increase in mean platelet volume with increasing sampling to processing interval is consistent with a number of other studies (Jackson and Carter, 1993; Wynn et al, 1995).

9.2.14 Determining the cut-off for reliability

The upward trend in the mean platelet volume only became significant after 4 days, and the trends in the haematocrit, mean red blood cell volume and monocyte count after 2 days. The data was therefore restricted accordingly. The neutrophil count was the least stable with age and the effect, although small, persisted even in the set restricted to 2 days – as did the effect on the total white cell count as the biggest contribution to this is from the neutrophil count.

Overall, given the necessary logistical restrictions when the study was set up, the findings of the stability analysis were better than expected and indicated that the results could be interpreted with confidence.

9.2.15 Association between postnatal age and red blood cell parameters

Although postnatal age is recognised as an important factor affecting haematological parameters in the general population, there are no previous reports of the effect of postnatal age on the haematological parameters in DS within the neonatal period. The large retrospective study only considered samples taken in the first week of life (Henry et al, 2007),
while the small prospective study examined samples from the first week of life, but did not take further samples until 6 weeks of age (Kivivuori, Rajantie and Siimes, 1996).

The association between increasing postnatal age and a decrease in haemoglobin, red blood cell count, haematocrit, mean cell haemoglobin and the nucleated red blood cell observed in this population of DS neonates is the same as that seen in the wider neonatal population (Vahlquist, 1941; Gairdner, Marks and Roscoe, 1951; Matoth, Zaizov and Varsano, 1971; Stockman and Oski, 1980; Perrone et al, 2005; Jopling et al, 2009; Christensen et al, 2011). Importantly, with regard to polycythaemia the fall in haemoglobin and haematocrit with increasing postnatal age meant that none met the criteria for polycythaemia by the fourth week of life.

The only red blood cell parameter not to show a significant change with increasing postnatal age was the mean red blood cell volume which remained fairly stable throughout the neonatal period. Studies in the general neonatal population have indicated a fall in the mean red blood cell volume with increasing age (Gairdner, Marks and Roscoe, 1951; Matoth, Zaizov and Varsano, 1971; Stockman and Oski, 1980). The small prospective study of DS neonates indicated that the mean red blood cell volume did fall from birth to 6 weeks and continued to do so until around 6 months of age. It may be that this was not evident in the CDSS as most samples were taken early in the postnatal period, or because the association becomes important after the neonatal period.

**9.2.16 Association between postnatal age and white blood cell parameters**

The pattern of change in the white blood cell count described here – a fall over the first two weeks and then a plateau – is again driven by that seen in the neutrophil component, and mirrors that seen in the wider neonatal population where it has been well documented (Kato, 1935; Xanthou, 1970; Manroet al, 1979; Schmutz et al, 2008). The only other white blood cell type to vary during the neonatal period is the basophil count which appears to fall throughout the neonatal period, but in a more linear fashion. Although, little has been published on the basophil count in this period there are some data indicating that the basophil count rises initially and then falls (Xanthou, 1970). Most of the literature on lymphocytes, monocytes and eosinophils suggests that the predominant pattern is for these to remain stable (Xanthou, 1970; Weinberg et al, 1985; Christensen et al, 2010), as seen in the CDSS population.
9.2.17 Association between postnatal age and platelet parameters

Again, the increase in platelet count seen throughout the neonatal period mirrors that consistently described in the wider neonatal population (Ablin et al, 1961; Aballi, Puaponhd and Desposito, 1968; Appleyard and Brinton, 1971; Arad et al, 1986; Kayiran et al, 2003; Ozyürek et al, 2006; Wiedmeier et al, 2009). This pattern is particularly important in the DS population who have a markedly lower platelet count at birth, with over 60% meeting conventional criteria for thrombocytopenia (platelet < 150 x 10^9/l). However, by the fourth week there were no cases meeting these criteria.
9.2.18 Summary of the neonatal analysis

The main findings of the neonatal CDSS analysis are of increased haemoglobin, haematocrit and mean red blood cell volume without an increase in the red cell count, an increase in and persistence of nucleated red blood cells, and thrombocytopenia with increased mean platelet volume. The effects of gestational age, birth weight and postnatal age are all similar to those described in the wider neonatal population with the striking exception of the relationship between gestational age and platelet count.

Some of these features are similar to those seen in fetal rather than neonatal haematopoiesis. In view of this and given the extraordinary occurrence of transient myeloproliferative syndrome in neonates with DS, which is thought to occur in a haematopoietic progenitor cell with a hepatic ontogeny, it is reasonable to hypothesise that the switch from hepatic to marrow haematopoiesis may be delayed in DS. However, taken together, the findings here are also consistent with the features of chronic fetal hypoxia. This idea is supported by the lower birth weight and earlier mean gestational age found in this population – and in other studies of DS neonates (Myrelid et al, 2002; Henry et al, 2007; Weijerman et al, 2008) - which are consistent with adverse intrauterine conditions and particularly with chronic fetal hypoxia. This is a different proposition altogether as this would appear to implicate an external factor – placental insufficiency - in contrast to the theory of delayed switching which may be considered as factor intrinsic to the fetus with DS. However, given that this is occurring in a neonatal population with DS a theory involving placental insufficiency would need to explain how the presence of DS affected the placenta in this way. There are two main possibilities: either there is a common maternal factor which is associated with placental insufficiency and DS; or, the presence of somatic trisomy 21 adversely affects the development or long term health of the placenta. Finally, it is possible that chronic fetal hypoxia may itself then lead to delayed switching from hepatic to marrow haematopoiesis.

Untangling the reasons for the haematological changes seen is important. If the changes relate entirely to intrinsic factors consequent on the presence of trisomy 21 then these will deepen our understanding of haematopoiesis. If there is also an extrinsic factor then the possibility of beneficial intervention becomes more realistic.

Historically it had been assumed that cerebral hypoxic-ischaemic injury in the neonatal population was primarily a consequence of acute peri-partum asphyxia. However, more recently it has become apparent that only a minority of cases have markers associated with acute asphyxia. Studies of children with cerebral palsy indicate that 10-20% only have
clinical or biological markers of acute perinatal or early neonatal asphyxia (Palmer et al, 1995; Hankins and Speer, 2003), and chronic fetal hypoxaemia is now thought to be causative in the majority. This has generated study into possible markers of chronic fetal hypoxaemia and an elevated nucleated red blood cell count has emerged as an important candidate.

In addition to its value as a potential marker of chronic intrauterine hypoxaemia, the nucleated red blood cell count may also be informative with regard to the timing of hypoxaemia. A study of the cord nucleated red blood cell counts of 153 neurologically impaired neonates were compared with those from 83 apparently healthy neonates (Korst et al, 1996). Clinical information was used to determine the likely timing of the hypoxic injury. The nucleated cell count was higher in those with neurological impairment. Importantly, within this group those with a history suggestive of chronic hypoxaemia had a higher nucleated red blood cell count, and these took longer to clear when compared with those who had a history suggestive of acute asphyxia. This finding is also consistent with a subsequent retrospective study of the full blood count results of 47 infants with asphyxia sufficient to cause subsequent neurological impairment (Phelan et al, 2007). The infants were grouped according to their clinical history. Those with apparent chronic intrauterine hypoxia appeared to have an elevated nucleated red blood cell count ≥26/100 WBC and/ or a platelet count <100 within the first 5 days of life - in contrast to those with a history consistent with acute birth hypoxia.

A study of the nucleated red blood cell count in term and AGA infants compared 29 infants with polycythaemia with a control group of 37 infants (Mandel et al, 2003). The haematocrit, red blood cell count and nucleated red blood cell count were all significantly higher and the platelet count was significantly lower in those with polycythaemia. The authors concluded that these features reflected intrauterine hypoxia.

Abnormal Doppler studies of the umbilical vein and artery are associated with adverse intrauterine conditions and intrauterine growth restriction. The relationship between abnormal Doppler studies of the umbilical artery, middle cerebral artery and umbilical vein, and nucleated red blood cell count was studied in 84 fetuses (Baschat et al, 1999). Increasing abnormalities of the Doppler studies were associated with an increasing nucleated red blood cell count and a longer time to clearance was seen in those with the most abnormal Doppler results. Similar results were observed in a study examining the effect of intrauterine hypoxia on the neonatal red blood cell count in 134 singleton pregnancies using Doppler studies of the fetoplacental circulation as a proxy of intrauterine hypoxaemia (Axt-Fliedner et al, 2001). Increasing abnormalities of the Doppler studies was associated with an increase in the
neonatal nucleated red blood cell count. This finding has also been replicated in SGA neonates (Bernstein, Minior and Divon, 1997).

It is clear that the constellation of polycythaemia, an elevated nucleated red cell count and its persistence in the peripheral circulation in the neonatal period, and thrombocytopenia are all consistent with chronic intrauterine hypoxia. The next consideration is to review evidence for a pathological mechanism to explain how and why this might occur in DS.

In the early development of the human placenta the trophoblast differentiates along two critical pathways: one to form the cytotrophoblast; one to form the syncytiotrophoblast. One of the essential functions of the latter is the synthesis of progesterone and human chorionic gonadotrophin (hCG). Although the literature on placental development in DS is limited, abnormal syncytiotrophoblast formation has been reported in DS (Frendo et al, 2001; Massin et al, 2001; Malassiné, Frendo and Evain-Brion, 2010; Pidoux et al, 2012). At least one of the underlying reasons for this would appear to be overexpression of SOD-1, which is located on chromosome 21, as overexpression has been demonstrated to lead to impairment of trophoblast fusion and differentiation (Frendo et al, 2000). Interestingly, in a recent paper, treatment with synthetic hCG has been shown to reverse abnormal trophoblast fusion and differentiation in vitro in a trisomy 21 affected trophoblast cell culture (Malassiné, Frendo and Evain-Brion, 2010).

There are various clinical features of placental insufficiency that may be picked up on routine ultrasonography. These include alterations in the fetal heart rate, a reduction in placental volume and absent or reversed end-diastolic flow – all of which have been reported in association with DS (Kariniemi and Aula, 1982; Metzenbauer et al, 2002; Wong and Levine, 2005). However, these do not appear to have been looked for in a systematic manner and the planned abstraction of the obstetric records associated with this population of DS neonates affords an opportunity for this to be done.

The second key finding here is of the altered relationship between gestational age and platelet count, namely a fall in platelet count with increasing gestational age in contrast to the relatively stable relationship described in the wider neonatal population. At first, this might suggest that there is a global increase in megakaryopoiesis in DS in utero which resolves towards term. However, this would not seem to be backed up by the platelet count which is demonstrably low. It does, though, appear to be a feature of an in utero process as the platelet count behaves after birth in the same way as in the wider population, increasing
throughout the neonatal period. This process may be driven by chronic hypoxia - discussed above as a possible cause for the distinct haematological profile of DS neonates. If there is indeed placental insufficiency then this would be expected to have an increasing impact with increasing gestational age and thrombocytopenia is a recognised feature of chronic intrauterine hypoxia. The haemoglobin, haematocrit and red blood cell count all increase with increasing gestational age as they might be expected to if their production is also being driven by hypoxia, but as this is also the direction of change in the general population it does not stand out.

Finally, in addition to elucidating further the mechanisms underlying the changes seen one of the next steps is to correlate the haematological findings in DS neonates with their clinical features to determine the clinical significance. In this regard there are three specific areas of immediate interest relating to polycythaemia, neutrophilia and thrombocytopenia. These are considered further in the final section on future work.
9.3 Blood cell morphology in DS neonates

This is the first report of the blood cell morphology of a prospective cohort of DS neonates using a validated morphology tool. Leading from the central hypothesis was the supposition that neonates with DS would have a distinct morphological profile, and that this might yield additional information that was not evident from the numerical parameters alone.

9.3.1 Development of a validated tool morphological analysis

One of the unique attributes of a haematologist is the continuity of care provided as both clinician and pathologist. In addition to their clinical duties, the haematologist is responsible for the interpretation of the numerical results and morphological appearances of blood and bone marrow samples. Although there is still progress to be made on understanding the significance of numerical parameters, particularly in children and in specific populations, these parameters are at least readily quantifiable. Morphological assessment, that is the study of the appearance or shape of blood cells, is far more qualitative. Indeed, for many the attraction and enjoyment of morphology is found in practising an art requiring skill, experience and a visual appreciation of subtle changes in colour or form.

The ability to interpret morphological appearance is largely taught by individuals to individuals and is further validated by the rigorous professional examinations that all haematologists are required to sit before being allowed to practice as a consultant. Recent research found there were 850 haematology consultants in the United Kingdom of whom fewer than 50 were paediatric haematologists (The British Society for Haematology and The Royal College of Pathologists, 2008). Consequently, expertise in haematological morphology is rare, with paediatric morphology an even rarer skill.

The attempt to standardise the morphology review was time consuming, and the failure of the initial proforma highlighted how qualitative morphology may be. The particular challenge of trying to standardise neonatal morphology reporting was described in a study designed to assess inter-reader variability in identification of segmented and immature neutrophils in 94 neonates (Schelonka et al, 1994). This concluded that ‘the clinical utility of the manual differential leukocyte count in the evaluation of neonates is limited’ because of the lack of agreement. In contrast, a comprehensive comparison of manual and automated results for neonates using the CellaVision DM automated microscope concluded that conventional microscopy remained essential when analysing neonatal samples (Billard et al. 2010).
The CellaVision is designed to determine a white blood cell differential by analysing the visual appearance of individual cells in far higher numbers than would be possible manually. It is also able to recognise a range of red blood cell abnormalities in the same way. The morphology reporting tool developed here was designed without any awareness of the CellaVision system, but is based on the same idea that each cell needs to be analysed and described. The validation exercise for the morphology reporting tool developed here indicated that there was good intra and inter-operator consistency, without which reassurance analysis would have been fruitless.

The results of the slides from the unselected neonatal population which were used in the morphology tool validation cannot be taken as reference data as the neonates were not healthy. Given the ethical difficulties in obtaining blood samples from healthy neonates who do not require venepuncture it will be difficult for reference data to be collected. Babies in the UK do have a heel prick test at day 5 for Guthrie spot testing and it might be possible to set up a study which involved a blood slide being made at the initial heel prick. It would potentially be possible for a large number of samples to be obtained in this way, but this was outside the scope of this present work.

9.3.2 Neonatal red blood cell morphology

The overall incidence of nucleated red blood cells was 59%. This increased from 49% for slides which were fully suitable to 60% for partially suitable and 65% for unsuitable slides. The fully suitable slides were taken at a mean age of 9.2 days, which was older than either of the other two groups. As the nucleated red blood cell count falls with increasing postnatal age the proportion would be expected to be lowest in the fully suitable group – as found here. Given that nucleated red blood cells should have disappeared by the fourth postnatal day, their persistence is striking and is consistent with the automated findings.

Spherocytes were present in just under half of all cases. Red blood cells are typically biconcave discs, in contrast spherocytes are spheroidal red blood cells which consequently lack an area of central pallor and are smaller in diameter with denser staining. They are associated with haemolytic states including hereditary spherocytosis, autoimmune and alloimmune haemolytic anaemia and hyposplenism. Howell-Jolly bodies are dark purple spheroidal or oblong inclusions, which are 1-2 µm across. They are nuclear remnants and are seen in any process causing erythroid hyperproliferation, including megaloblastic anaemia, thalassaemia and hyposplenism. Spherocytes and Howell-Jolly bodies are recognised as features of neonatal blood films, where it is assumed that they are a feature of hyposplenism.
(De Alarcón and Werner, 2004). Given the lack of reference data it is not possible to say whether their presence here simply reflects that of a neonatal population, or whether there is an increased erythropoietic drive.

Target cells were present in a third of cases. Target cells are associated with haemoglobinopathies, thalassaemia, iron deficiency, liver disease and hyposplenism. However, they may also be seen as an artefact of rapid drying when the haemoglobin tends to ‘puddle’ in the centre of large cells and so their presence has to be interpreted with caution.

Although basophilic stippling was uncommon, occurring in around 10% cases, this was still thought to be higher than expected. Basophilic stippling refers to the presence of fine blue grey stipple which represent clustered ribosomes in immature red blood cells. They are a marker of ineffective erythropoiesis and are seen in many erythropoietic disorders with the exception of iron deficiency anaemia. They are increased in thalassaemia, megaloblastic anaemias, unstable haemoglobins, haemolytic anaemia, dyserythropoietic states, liver disease, heavy metal poisoning and pyrimidine 5'-nucleotidase deficiency.

Taken together, the main findings here are of persistent nucleated red blood cells, which may reflect intrauterine hypoxia (as discussed in section 9.2.18) and a constellation of red blood cell features associated with hyposplenism and increased erythropoiesis.

### 9.3.3 Neonatal neutrophil features

The additional information contributed by the individual cell analysis was very helpful in indicating how common a feature was when it was present. For example, both agranular neutrophils and hypersegmented neutrophils were present in 7/49 (14%) slides. However, ~7% neutrophils were agranular while only 0.5% neutrophil were hypersegmented indicating that even when the latter were present they were rare. Hypersegmented neutrophils occur when there are five or more nuclear lobes and are an indication of megaloblastic anaemia or of a severe haemolytic anaemia. They are occasionally features of chronic infection, chronic myeloid leukaemia, myeloproliferative disorders and of cytotoxic or anti-metabolite therapy. The frequency here does not appear remarkable.

In contrast, the finding of agranular neutrophils does appear to be significant, especially when the very high frequency of hypogranular neutrophils is taken into account. These were seen in almost all slides. Overall, just under half of all neutrophils had reduced granularity - 40% being hypogranular and 7% agranular. Hypogranular neutrophils lack the small pink granules
usually seen in mature neutrophils, or the coarse purple primary granules seen in toxic granulation, and are a feature of myelodysplasia (Widell et al, 1995; Germing et al, 2012).

Neutrophil vacuolation was common, being seen in just over three quarters of slides and a quarter of neutrophils. Vacuolation may be associated with metabolic disorders – both in those with the disease and occasionally in heterozygotes. It is postulated that it occurs as a result of dissolution of abnormal inclusions. Vacuolation is also seen in infection, ethanol ingestion, Jordan’s anomaly where the vacuoles contain lipid (Rozenszajn et al, 1966). Neutrophil vacuolation has also been described in grey platelet syndrome (White and Brunning, 2004).

Pelgeroid neutrophils were also common, being present in just over half of slides, and around 10% of neutrophils. Pelgeroid neutrophils are described when there is a bilobed nucleus and coarsely clumped chromatin. This combination is referred to as the Pelger-Huët anomaly, having been first described by Pelger in 1928 and the familial nature recognised by Huët in 1931 (Cunningham et al, 2009). The anomaly may be acquired in acute or chronic myeloid leukaemia, myelodysplasia or after cytotoxic chemotherapy. In comparison with the constitutional form in pseudo Pelger-Huët anomaly the bilobed cells are a minority, as here. Pelgeroid neutrophils can be seen when there is a delay in processing, as can vacuolation (Anderson et al, 2005), and although this may account for some of the incidences it should not account for all as only those with intact morphology were selected.

9.3.4 Neonatal platelet morphology

Giant platelets and/or megakaryocyte fragments were ubiquitous. Giant platelets are platelets with a diameter similar to that of a red blood cell or small lymphocyte. Aside from rare congenital syndromes, they are often a feature of increased turnover, and are seen in immune thrombocytopenia, thrombotic thrombocytopenia, disseminated intravascular coagulation, myeloproliferative disorders (including acute megakaryoblastic leukaemia), myelodysplasia, hyposplenism and megaloblastic anaemia. There is no formal definition of a megakaryocyte fragment, and so the following advice was followed: “It is rather arbitrary how you draw a dividing line between a giant platelet and a megakaryocyte fragment. If a giant platelet has a normal structure, i.e. a central granulomere and a peripheral hyalomere, I am happy to call it a giant platelet regardless of its size. If it is structurally abnormal and really enormous I think one could reasonably use the term ‘megakaryocyte fragment’. I think one sees such things in myeloproliferative neoplasms in accelerated phase.” (Barbara Bain; personal communication).
In addition to the increase in platelet size, the platelet colour was abnormal, being pale in around two thirds of cases. Platelet colour is mainly due to small azurophilic alpha granules. If these are reduced or absent then the platelet appears pale blue or grey as in grey platelet syndrome where there is loss of alpha granules during megakaryocyte maturation with consequent poor aggregation and a bleeding diathesis. In contrast platelet colour may also appear pale where there have been granules, but where these have been released either in vivo or in vitro. The former may occur after cardiopulmonary bypass and the latter after difficult venepuncture when the platelets may also be clumped. Agranular platelets are also seen in myelodysplasia. There is no reason to suppose that venepuncture was especially difficult and the pale colour was not associated with platelet clumps.

Megakaryocytes and bare nuclei were also seen, but as discussed in section 2.2.3 these are recognised features of neonatal films.

Taken together, both the increase in platelet size and the pallor may indicate an underlying myeloproliferative/myelodysplastic process.

### 9.3.5 Blast cells in the neonatal films

Overall, blasts were present in around half of all the neonatal blood films. When this is broken down by suitability of the slide the proportion of slides with blasts identified falls from 56.9% for those with suitable slides, to 52.8% for those with only partially suitable slides and then to 37.9% for those with the poorest quality slides. This apparent fall is likely to be because the blast cell morphology becomes harder to recognise as the morphology deteriorates and so is under-reported in those classed as ‘unsuitable’. When there is a delay in processing a blood sample artefactual changes may appear which can mimic blasts as the nucleoli become more pronounced and the grainy appearance of the nucleus becomes smoother and glassy. There had to be at least 3 cells with an unequivocal appearance of a blast for blasts to be deemed present. It was encouraging that the suitability status did not change on re-review for any of the slides.

The best evidence suggests that the incidence of TMD in neonates with DS is probably around 4% (Pine et al, 2007). It is therefore clear that the presence of blasts alone does not indicate TMD. However, there are distinct morphological features which suggest TMD – particularly the appearance and number of blasts. In the CDSS analysis a diagnosis of TMD, based on morphological appearances, was made in 8/189 (4.2%) cases and this incidence would be consistent with other work.
9.3.6 **Comparison of manual and automated methods for identifying blasts**

One of the questions being addressed here is whether manual examination of a blood film is required to identify TMD. It is clear from the above that blasts are common and that review of all films with blasts is required to differentiate those with the appearances of TMD. The next question is whether the automated analysis was able to identify accurately which samples had blasts – in which case it could be argued that films only needed to be made on these. In fact, when the blast flags only were considered the sensitivity was only 72%, and although this improved to 90% when all abnormal white blood cell flags were used this is still inadequate. Moreover, the sensitivity was lower when the ability of the machine to identify blast in films with TMD was considered, and even when all abnormal white cell flags were considered 25% samples with TMD had no flags.

The relatively low sensitivity and specificity are at first surprising, but are actually quite similar to results obtained in a performance evaluation of the Sysmex XE-2100 which looked at the immature granulocyte flag (Briggs et al, 1999). This evaluation did not consider the blast flag.

9.3.7 **Summary of neonatal morphology findings**

The morphology review of neonatal blood films in the CDSS demonstrated that blood films from neonates in the CDSS appear to have distinct features. In particular, there were features of:

1. Hyposplenism and increased erythropoiesis - spherocytes, Howell-Jolly bodies, target cells, basophilic stippling and nucleated red blood cells;
2. chronic placental insufficiency - persistence of nucleated red blood cells with giant platelets;
3. myelodysplasia/myeloproliferation - hypogranular neutrophils, Pelgeroid neutrophils, giant platelet and platelet pallor.

Moreover, comparison of the automated analysis and manual reviews indicated that the former was unable to identify all samples with blasts and given the clinical imperative to identify TMD this research suggests that morphological review of a blood film is indicated in all neonates with DS.
9.4 Haematological values beyond the neonatal stage

9.4.1 Introduction

This section considers the results of the analysis of haematological parameters in children with DS beyond the neonatal period.

Considerable thought was given to the best way to present this data. When children were beyond the neonatal period, samples tended to arrive at any point in the year with no specific relationship to their birthday. It would have been possible to create simple scatter graphs with each result plotted and these can be useful ways to consider change with time. However, one of the driving forces of this work has been the desire to produce results in a way that might be clinically useful. It was therefore decided to consider results around each birthday, accepting that samples taken 3-9 months after a birthday would be lost from the analysis. This meant that the mean values for each year could be developed. Although some groups have included multiple results for the same child this was not done here in order to avoid bias.

9.4.2 Red blood cells

One of the key findings is that there is a distinct change in the haematological profile in this population between birth and 1 year. The haematological parameters are then fairly stable throughout the period examined, although there appears to be a slight trend upwards in the haemoglobin and mean cell haemoglobin. There are several previous studies of children with Down syndrome which have considered red cell parameters. However, none of these included neonates and so the change in parameters over the first year of life has not been described in the same population before. The second important finding is that children with DS appear to have a distinct haematological profile with an increase in haemoglobin, mean cell haemoglobin, haematocrit and mean cell volume.

Red blood cell count

The mean red cell count in the 1-4 year old children was 4.5 (4.4, 4.7) x10^{12} /l. There are two previous reports of red cell count in children with DS, both of which reported similar values. The first looked at 63 children aged 3 months to 7 years and reported a red cell count of 4.4 ± 0.4 x10^{12} /l (mean ± SD) in those aged 3 months to 2 years and 4.3 ± 0.1 x10^{12} /l (mean ± SD) in those aged between 2-7 years (Starc, 1992). The second considered 17 children aged between 2-5 years old and reported a red cell count of 4.7 ± 0.3 x10^{12} /l (mean ± SD) (David et al, 1996).
When the values obtained here for children with DS are compared with two sets of reference data for the wider paediatric population they also appear to be similar. The first dataset is compiled from several sources and is provided in the most recent edition of a classic paediatric haematology reference book (Orkin and Nathan, 2009) and the second is the “in house” paediatric reference range developed by the York Hospital haematology laboratory (Martin Howard and Martin Skelton, personal communication). These give a red cell count of $4.5 \pm 0.8 \times 10^{12} / l$ (mean ± 2SD) for children 6 months to 2 years and of $4.6 \pm 0.7 \times 10^{12} / l$ (mean ± 2SD) for children aged 2 to 6 years, or a red cell count range of 3.9-5.3 $\times 10^{12} / l$ for children aged 2-6 years respectively. The values for children with DS in the CDSS are consistent with these. Moreover, the two studies which reported the red cell count in DS also included age matched control groups (Starc, 1992; David et al, 1996). There was no difference in the red cell count in children with DS compared with controls in these reports, consistent with these findings.

Haemoglobin

However, although the haemoglobin falls after birth it still appears to be higher than in the wider paediatric population. There are three studies which report the haemoglobin in children with DS – the two above which considered red cell count (Starc, 1992; David et al, 1996), and a recent paper designed to investigate the prevalence of iron deficiency in DS (Dixon et al, 2010). Although the age range of children studied in the latter paper was 1 to 19.7 years, the median age was only 4.7 years and so it is a comparable population. These studies reported a haemoglobin of 12.8 ± 1 g/dl (mean ± SD) in those aged 3 months to 2 years and 12.9 ± 0.8 g/dl (mean ± SD) in those aged between 2-7 years (Starc, 1992); 13.5 ± 1.3 g/dl (mean ± SD) in children 2-5 years old (David et al, 1996); and 13.1 ± 1.1 g/dl (mean ± SD) in those aged 12 to 36 months and 13.8 ± 1 g/dl (mean ± SD) in those over 36 months (Dixon et al, 2010). These are all similar to the values obtained in the CDSS population. Moreover, there appears to be a slight trend upwards in the haemoglobin count after 2 years of age which is consistent with the findings of the most recent study. This appears to be driven by an increase in mean cell haemoglobin as the red cell count is stable.

The two reference datasets used here give a haemoglobin of 12 ± 1.5 g/dl (mean ± 2SD) in those aged 6 months to 2 years and 12.5 ± 1 g/dl (mean ± 2SD) in those 2 to 6 years old or a range of 11.5-13.5 g/dl. Although there is some overlap with the values obtained in the CDSS, it is evident that the values for the DS population are higher - for example, the mean haemoglobin for children with DS aged 3 years was 13.5 g/dl which would be right at the top
of both the textbook and the hospital range. Further the upper limit of the range for haemoglobin values in the CDSS for children at their 1\textsuperscript{st} birthday and beyond is ≥ 14 g/dl which is above the upper limit of the reference range. The two papers which included age matched control groups (Starc, 1992; David et al, 1996) found a significant increase in the haemoglobin in DS compared with controls, consistent with these findings.

Mean cell haemoglobin

Mean cell haemoglobin has been reported in children with DS in the same three studies which considered haemoglobin (Starc, 1992; David et al, 1996; Dixon et al, 2010). These reported a mean cell haemoglobin of 29.5 ± 2 pg (mean ± SD) in those aged 3 months to 2 years and 29.9 ± 1.5 pg (mean ± SD) in those aged between 2-7 years (Starc, 1992); 29.1 ± 1.6 pg (mean ± SD) in children 2-5 years old (David et al, 1996); and 29.2 ± 1.5 pg (mean ± SD) in those aged 12 to 36 months and 30.7 ± 1.8 pg (mean ± SD) in those over 36 months (Dixon et al, 2010). These are all similar to the values obtained in the CDSS population. Moreover, there again appears to be a slight trend upwards in the mean cell haemoglobin count after 2 years of age which is consistent with the findings of the most recent study which leads to an apparent increase in haemoglobin.

The only reference values indicate a mean cell haemoglobin of 27 ± 4 pg (mean ± 2SD) in those aged 6 months to 2 years and 27 ± 3 pg (mean ± 2SD) in those 2 to 6 years old (Orkin and Nathan, 2009). Although there is some overlap with these values and the CDSS results, the SD for the wider paediatric population is fairly large. When the mean values are considered the mean cell haemoglobin in DS appears higher, and again it can be seen that the upper limit of the range for mean cell haemoglobin at and beyond the 1\textsuperscript{st} birthday is ≥ 31.5 pg, which is above the upper limit for the reference population. The two papers which included age matched control groups also found a significant increase in the mean cell haemoglobin in DS compared with controls (Starc, 1992; David et al, 1996).

Haematocrit

Haematocrit has been reported in children with DS in four studies (Roizen and Amarose, 1993; Starc, 1992; Bartelik, 1995; David et al, 1996). These reported a haematocrit of 39.1 % (range 35.8-41.8) in those aged 2-6 years (Roizen and Amarose, 1993); 38.4 ± 3.2 % (mean ± SD) in those aged 3 months to 2 years and 38.8 ± 1.7 % (mean ± SD) in those aged between 2-7 years (Starc, 1992); and 40.2 ± 2.6 % (mean ± SD) in children 2-5 years old (David et al, 1996). These are all similar to the values obtained in the CDSS population. Although there is an increased incidence of congenital cardiac defects in DS these are not
usually cyanotic. The NDSP reported an incidence of major cardiac defects in 44% neonates with DS, with ventricular septal defect in 43%, secundum atrial septal defect in 42%, atroventricular septal defect in 39% and tetralogy of Fallot in 6% (Freeman et al, 2008). Only the latter is cause of cyanotic heart disease and so is unlikely to account for the increase seen in haematocrit.

The reference values give a haematocrit of 36 ± 3 % (mean ± 2SD) in those aged 6 months to 2 years and 37 ± 3 % (mean ± 2SD) in those 2 to 6 years old (Orkin and Nathan, 2009); or a local hospital range of 34-40 %. There is no overlap with the mean values for DS obtained here for children aged 1, 2 or 3 years old consistent with a significant difference. For those aged 4 years old the mean haematocrit was 40 (39, 41) % (mean; 95% CI). Again the upper limit of the range for haematocrit in the CDSS at and beyond the 1st birthday was ≥ 42% which was above the range for the wider paediatric population. This is in agreement with three case-controlled studies which found that haematocrit was significantly increased in children with DS (Roizen and Amarose, 1993; Starc, 1992; David et al, 1996).

Mean cell volume

Mean cell volume has been reported in children with DS in five studies (Roizen and Amarose, 1993; Akin, 1988; Starc, 1992; Bartelik, 1995; David et al, 1996). These reported a mean cell volume of 86.9 (81.8, 97.8) fl in those aged 2-6 years (Roizen and Amarose, 1993); mean 90 fl in 7 children whose age was not reported (Akin, 1988); 87.9 ± 6 fl (mean ± SD) in those aged 3 months to 2 years and 89.6 ± 3.4 fl (mean ± SD) in those aged between 2-7 years (Starc, 1992); and 86.5 ± 4.6 fl (mean ± SD) in children 2-5 years old (David et al, 1996). These all overlap the values obtained in the CDSS population, although the values obtained in the CDSS appear slightly higher.

The reference values give a mean cell volume of 78 ± 8 fl (mean ± 2SD) in those aged 6 months to 2 years and 81 ± 6 fl (mean ± 2SD) in those 2 to 6 years old (Orkin and Nathan, 2009); or a local hospital range of 75-87 fl. There is no overlap with the mean values for DS obtained here for children aged 1, 2 or 3 years old consistent with a significant difference. For those aged 4 years old the mean cell volume was 89.4 (85.1, 93.6) % (mean; 95% CI). The mean value for mean red cell volume in DS is outside the ranges for the wider paediatric population. The upper limits of ranges for the children in the CDSS appear to fall with increasing age, but even at the 4th birthday point the upper limit is 95.5 fl which is considerably higher than that of the reference population. This is in agreement with three
studies which were case-controlled studies which found that mean cell volume was significantly increased in children with DS (Starc, 1992; David et al, 1996).

The persistence of an increased mean cell volume has important clinical implications. A child with DS and iron deficiency might have a mean red cell volume which appeared normal when compared to standard reference charts. The iron deficiency might be missed and remain untreated with adverse health consequences. This concern has recently been substantiated by a study of 114 children with Down’s syndrome which set out to determine the prevalence of iron deficiency in this population (Dixon et al, 2010). It found that 13/15 children who were diagnosed with iron deficiency or iron deficiency anaemia would have been missed if red blood cell indices alone had been used as a means of screening as a result of the elevated mean red cell volume in this group. Alternatively, unnecessary blood samples might be taken to investigate an apparently high mean red cell volume, when it was actually within the normal range for DS.

Nucleated red blood cells

Nucleated red blood cells are expected to disappear from the blood after four days, although it has already been noted in the neonatal analysis that there is a tendency for them to persist in neonates with DS. The finding that nucleated red blood cells are present in the peripheral blood at 1 year in the CDSS population is in stark contrast to the wider paediatric population where they would not be expected in healthy children. They are seen when there is an increase in erythropoiesis or in association with bone marrow infiltration. There are many potential causes of increased erythropoiesis, one of which is iron deficiency. The prevalence of iron deficiency in DS has been reported as 10% (Dixon et al, 2010), which is broadly similar to prevalence estimates in the wider paediatric population (Rodd and Blankenstein, 1995; Cogswell et al, 2009) suggesting that this does not wholly account for the presence of nucleated red blood cells in the 1 year old group which appears to be a feature of DS. Interestingly, the prevalence appears to be almost twice as high in those 12-36 months compared with those > 36 months old (Dixon et al, 2010), or in those 1-2 year old compared with those 3-5 years old (Cogswell et al, 2009). Given that nucleated red blood cells were only seen in those at 1 year, and not a 2, 3 or 4 years, when iron deficiency is less likely to occur, it may have a role in their presence.

In summary, it appears that the haemoglobin, mean cell haemoglobin, haematocrit and mean cell volume are higher in children with DS while the red cell count is similar to that of the general paediatric population.
Analysis of the white blood cell parameters again indicates that there is a marked change between parameters at birth and those taken around subsequent anniversaries, which appear to be fairly similar. Moreover, the white blood cell count appears to be lower when compared with the wider paediatric population and this is driven particularly by a lower lymphocyte count.

*Total white blood cell count*

A low white blood cell count in apparently healthy children with DS was reported in a longitudinal study of 8 children being followed up to determine the effects of growth hormone in this group (Ridler and Shapiro, 1959). A preliminary study of 7 children with DS reported a mean white cell count of 6.2 x10\(^9\)/l which it compared with an expected range of 7-13 x10\(^9\)/l (Akin, 1988). A subsequent case controlled study looking at 18 children aged 2-6 years reported the white cell count in 14/18 (Roizen and Amarose, 1993). Although the mean white cell count was lower in the children with DS at 6.6 (3.3-10) x10\(^9\)/l (mean; range) compared with the controls at 8.2 (5.4-15.1) x10\(^9\)/l (mean; range) this was not significant. Following this there have been three further case controlled studies. A study of 62 children with DS aged 3 months to 10 years, along with a control group of 25 children, reported a lower white cell count in those with DS (Bartelik, 1995). A study of 17 children with DS aged 2-5 years found a lower white cell count in those with DS at 6.8 ± 2.4 x10\(^9\)/l (mean ± SD) compared with 8 ± 1.4 x10\(^9\)/l (mean ± SD) in 23 age-matched controls (David et al, 1996). The most recent study looked at 41 children with DS and again reported a lower white cell count compared with 41 age matched controls (Bloemers et al, 2010a). The mean white cell counts in all these studies are similar to those reported here for the CDSS population where it ranged between 3.4-10.7 x10\(^9\)/l for those aged 1-4 years.

The reference values give a mean white cell count of 10.6 (6-17) x10\(^9\)/l (mean; 95% CI) in 2 year olds and 9.1 (5.5-15.5) x10\(^9\)/l (mean; 95% CI) in 4 year olds (Orkin and Nathan, 2009); or a local hospital range of 5-17 x10\(^9\)/l for 2-6 year olds. Although most of the mean values obtained for the children with DS overlap with these, they are all at the lower end of the reference ranges, and the mean white cell count for DS children at 2 years is outside the reference range. Moreover, it can be seen that the lower end of the ranges for the CDSS population at and beyond the 1\(^{st}\) birthday is lower with a minimum value of 3.4 x 10\(^9\)/l, while the upper end is lower than the wider paediatric population with a maximum value of 10.7 x 10\(^9\)/l.
Only a few studies have also looked at the white blood cell differential in children with DS. These were all case controlled and all agreed that there appears to be a lower lymphocyte count (Bartelik, 1995; David et al, 1996; Bloemers et al, 2010a). Bloemers, who used flow cytometry, also reported lower absolute neutrophil and monocyte counts, but with a 1.5 fold increase in CD14(dim)CD16(+) monocytes. These are proinflammatory and they speculate that they may play a role in the many chronic inflammatory conditions associated with DS.

Separately, an increase in ALL and early infections are both associated with DS. However, although population-based studies have identified an increased morbidity and mortality due to infection (Hill et al, 2003), there are no detailed reports of the infection pattern of a cohort of DS children – although a recent prospective cohort study did demonstrate that DS is a risk factor for respiratory syncitial virus related bronchiolitis (Bloemers et al, 2007). However, although studies have suggested that there is an altered lymphocyte profile in DS, the basis of any immune defect remains unclear (Hill et al, 2003; Ram and Chinen, 2011; Joshi et al, 2011). Co-existent physical problems may also exacerbate a susceptibility to infection – for example, congenital cardiac defects predisposing to endocarditis (these may require surgery with an increased risk of nosocomial infection); narrowing of the Eustachian tube predisposing to persistent otitis media with effusion (glue ear); feeding difficulties with an increased risk of aspiration and consequent respiratory problems; and an increase in mouth breathing in DS predisposing to respiratory infection. The pattern of infections in children with DS merits further investigation, and may shed light on the apparent predisposition to infection to help target further immunological evaluation of this population.

When the ranges for the differential counts are considered it becomes apparent that the lower limit changes relatively little even from the values at birth, while the upper limit falls in the period from birth to the 1st birthday and then stays relatively stable thereafter.

The mean neutrophil counts in the CDSS at 2 and 4 years are 2.4 x 10^9/l and 4.1 x 10^9/l respectively which appear similar to the reference values of 3.5 x 10^9/l and 3.8 x 10^9/l at the same ages (Orkin and Nathan, 2009). The mean lymphocyte counts at 2 and 4 years were 2.3 x 10^9/l and 2.2 x 10^9/l which appear lower than the reference values of 6.3 x 10^9/l and 4.5 x 10^9/l at the same ages (Orkin and Nathan, 2009). The mean monocyte counts at 2 and 4 years were 0.4 x 10^9/l and 0.5 x 10^9/l which appear similar to reference values of 0.5 x 10^9/l and 0.5 x 10^9/l at the same ages (Orkin and Nathan, 2009). The mean eosinophil counts at 2 and 4 years were 0.13 x 10^9/l and 0.15 x 10^9/l which appear similar to reference values of
0.3 x 10^9/l and 0.3 x 10^9/l at the same ages (Orkin and Nathan, 2009). There are no appropriate reference values for basophil counts in children.

Overall, it appears that the white cell count and the lymphocyte count are lower in children with DS compared with the wider paediatric population.

### 9.4.4 Platelets

Although thrombocytopenia is a feature of DS during the neonatal period the platelet count has been shown to rise with postnatal age. It appears that the platelet count is similar to that of the wider paediatric population during the four years studies here.

There is just one case controlled study of the platelet count in children with DS (David et al, 1996). This reported a mean platelet count of 326 ± 54 x 10^9/l (mean ± SD) in children aged 2-5 years with DS compared with 324 ± 69 x 10^9/l (mean ± SD) in age matched controls. There was no significant difference between these. The values obtained in the CDSS population, with means of 278-308 x 10^9/l, also appear similar.

There is little reference data for platelet counts in the wider paediatric population. There is some data from a study intended to generate reference thrombopoietin values for children (Ishiguro et al, 1999) These give values of 314 ± 78 x 10^9/l (mean ± SD) and 304 ± 66 (mean ± SD) at 1-2 years and 3-4 years which are similar to the values obtained in the DS children here.

There is also little data on the mean platelet volume in children. The same case controlled study mentioned above did look at this parameter and found no significant difference between cases and controls with mean platelet volume 7.3 ± 0.8 fl (mean ± SD) in children aged 2-5 years with DS compared with 8 ± 1.1 fl (mean ± SD) (David et al, 1996). Although the mean platelet volume fell between birth and 1 year, with stable values thereafter, the mean values obtained in the CDSS appear to be higher than either the children with DS or the wider paediatric population with means of 10.3-10.5 fl during the 4 years studied.
9.4.5 Summary of the haematological analysis beyond the neonatal stage

The analysis of haematological parameters in children in the CDSS beyond the neonatal period shows that although there is a marked change between birth and the 1st birthday, with most parameters being fairly stable thereafter, they do not “normalise” and children with DS appear to have a distinct haematological profile throughout their early years. Children in the CDSS had higher haemoglobin, mean cell haemoglobin, haematocrit and mean cell volume, but a lower white blood cell count, when compared with accepted reference values. The lower white cell count appears to reflect a lower lymphocyte count. In contrast to the differences seen in the neonatal period, the platelet count was similar to that of the wider paediatric population.
9.5 Establishment of the cohort

9.5.1 Points arising from the consultation process

The consultations conducted as part of the scoping work confirmed that there was a willingness to participate – both from clinicians and families - and this lent momentum to the development of the study. It did however reveal a striking variety of practice, which needed to be accommodated within the study design. In particular, there were several points of practice which emerged from the consultation phase and which were important in shaping the study.

Taking a full blood count in the neonatal period

It became evident that it was not routine practice to check a full blood count in all neonates with DS. This was unexpected for several reasons: anecdotally, the paediatric haematologists consulted thought that they were often asked to review and full blood count results from DS neonates, and also, they believed it was good practice to check a full blood count in DS neonates.

As described already, children with DS are at an increased risk of ALL and AML. In children with DS and a history of TMD the risk of acute leukaemia, is even higher. Although fatal if untreated, ML-DS has an excellent response to treatment, and occurs within a relatively short period of time – the first five years of life. In the local Regional Centre for Paediatric Haematology it has been considered good practice therefore to monitor children with a diagnosis of TMD during this period of increased risk. The monitoring would typically consist of three monthly full blood counts during the first two years and then six monthly full blood counts, all with morphology review, up to five years of age. In addition, the diagnosis of TMD would be recorded in the medical notes. If a child who is known to have had TMD develops a cytopenia then the child is investigated promptly given the particularly high risk of early acute leukaemia.

Once it was realised that there was a discrepancy between what was perceived to be good practice and what was actually happening wider consultations were initiated. These involved paediatric haematologists in other centres. There was a consensus amongst those questioned that current understanding of the relationship between neonatal TMD and subsequent ML-DS had developed such that it should now be considered good practice to check full blood counts routinely in all neonates with DS. In fact, this was already
recommended practice in other countries (Van Cleve and Cohen, 2006; Henry et al, 2007).
The situation was then reviewed with the clinicians on the Stakeholder Committee, and with
neonatologists in the YNN. It was then agreed that a new service would be offered by the
Regional Centre for Paediatric Haematology to hospitals within the region: if clinicians wished
to have a full blood count and morphology reviewed by a paediatric haematologist then they
could send a sample directly to Regional Centre for Paediatric Haematology. This was a new
clinical service developed in response to this perceived need, independent of the study.

Taking blood tests during the early years

It became clear that the recommended annual blood tests were done less frequently in actual
practice. This meant that there were fewer samples from beyond the postnatal period.

Relationship with stakeholders

The relationships with the DSA and the DSMIG were foundational in the study development
process. The contact with both was initially to seek advice, but in both cases then developed
in one of active support from the organisation for the CDSS. The DSA invited the CDSS to
submit an article about the study for publication in the quarterly journal it produces for
members (Appendix 10). They also volunteered to include information about the CDSS in the
packs sent out to all new members of the DSA. The latter offer was not taken up, largely
because of the resource implications.

9.5.2 Hospital participation

One of the concerns of the CDSS was to recruit a population that was generalisable to the
wider population of neonates with DS. Consequently, all hospitals within the target area with
a maternity unit were approached, whether they were large teaching hospitals with a high
birth rate or smaller units in more rural locations with low birth rates. All hospitals agreed to
participate and almost all recruited into the CDSS. Recruitment was highest from the
Yorkshire Neonatal Network where the study was piloted and where there were already links
established with clinicians.
Chapter 10 Conclusion

10.1 Main findings

The main aim of this work was to describe the haematological profile of neonates with DS and to examine factors that might be associated with variability. It has been demonstrated that neonates with DS have an increased haemoglobin, haematocrit and mean red cell volume, without a marked increase in the red blood cell count, an increase in and persistence of nucleated red blood cells, and thrombocytopenia with increased mean platelet volume. Taken together these features are consistent with chronic intrauterine hypoxia. Recent research on trophoblast formation in trisomy 21 supports this suggestion.

The effects of gestational age, birth weight and postnatal age on haematological parameters are all similar to those described in the wider neonatal population with the striking exception of the relationship between gestational age and platelet count. In the wider population the platelet count remains stable with increasing gestational age. In contrast, in the CDSS the platelet count fell with increasing gestational age.

The analysis of the morphology of neonates with DS demonstrated features consistent with chronic intrauterine hypoxia, hyposplenism as well as increased, and possibly ineffective, erythropoiesis. Further, some features are consistent with a myeloproliferative/myelodysplastic process. Moreover, this work demonstrated that automated analysis was not sufficient to identify blasts and manual review of a film is indicated to look for evidence of TMD.

Analysis of the haematological parameters in children beyond the neonatal period and up to their 4th birthday demonstrated a change in parameters between birth and the 1st birthday with most parameters being fairly stable thereafter. It was also demonstrated that the haematological profile continues to differ from that of the wider population with increase in haemoglobin, mean cell haemoglobin, haematocrit and mean cell volume. In contrast, the white blood cell count appears to be lower, largely as a consequence of a lower lymphocyte count. The platelet count appeared to be similar to that of the wider paediatric population during the four years studied.
10.2 Significant outcomes

Establishment of the CDSS

One of the most significant outcomes of this work is the successful establishment of a cohort of neonates with DS. This was developed from scratch, involving considerable time and effort.

Most large cohort studies are voluntary and consequently true population based studies are rare. The CDSS relied on the voluntary participation of hospitals, clinicians and families and thus is not truly population based although it is the closest to a population based birth cohort of children with DS that there has been. The aim here was to develop a cohort within which the haematological parameters of neonates particularly could be examined, and this has been done.

One of the accepted limitations of the study is that there is little information on those who did not take part. The British Paediatric Surveillance Unit (BPSU), which comes under the auspices of the Royal College of Paediatrics and Child Health, requires clinicians to notify the BPSU at regular intervals every time certain named diagnoses are made. However, this list does not include a diagnosis of DS. Data on diagnoses of DS is available from the National Down Syndrome Cytogenetic Registry (NDSCR). Regional cytogenetic laboratories voluntarily notify the NDSCR when they make a diagnosis of DS. The majority of these are prenatal diagnoses. The NDSCR estimates their ascertainment at ~94% and the live birth rate for DS has consistently been at 1.1: 1000 live births during the study period. However, as the purpose of the NDSCR is to describe trends in the numbers of Down’s syndrome live births and antenatal diagnoses in England and Wales it records very few identifying details. There is therefore no complete register of children born with DS in the UK. Further research in this area would undoubtedly be facilitated by the establishment of a National Registry such as exist for cancer, which was able to record more complete demographic information.

Moreover, in the UK the number of children born with DS has remained relatively stable over the last twenty years, at just over one in every 1000 live-births. Any fall in births that might be expected from an expanded antenatal screening programme appears to have been offset by a rise in maternal age (Morris and Alberman, 2009). Therefore the needs of this population remain as important as they have always been.

The important question for the CDSS is whether the fact that it was voluntary and not population based has significantly influenced the results. As discussed in section 9.1 the
case characteristics for the CDSS are comparable to what would be expected and this suggests that the results are generalisable.

Dissemination of information

During this process information about the CDSS was disseminated in a number of ways – primarily through direct contact with clinicians across the north of England, but also through the website (www.cdss.org.uk), oral and poster presentations to the DSMIG, national and international scientific meetings, and through publications in the Journal of the DSA and in peer reviewed journals (James et al, 2008; James and Kinsey, 2009). Further details of these are provided in Appendices 10-19.

Change in clinical practice

When this work was begun it was not routine practice to take a full blood count in neonates with DS. This practice changed during the course of the study as awareness of the significance of TMD increased - often as direct outcome of presentations and discussions about the CDSS. For example, the Leeds Teaching Hospitals NHS Trust guidelines for the management of a neonate with DS were revised to recommend that all neonates with DS have a full blood count and film (available on the Leeds Teaching Hospitals Trust intranet: www.lhp.leedsth.nhs.uk). In addition, the national recommendations of the DSMIG have changed following presentations at national meetings (Charleton, Dennis and Marder, 2010) and revised guidelines are due to be published next year.

Characterisation of the neonatal haematological profile in DS

This work has all been carried out with clinical management in mind and it is hoped that the description of mean and range values for neonates, and older children, with DS will be a useful tool for clinicians.

Development of a validated morphology review tool

The initial morphology review highlighted a number of different problems with traditional ways of reporting morphology and required the development of a new tool for morphology reporting. This has been validated and successfully used in the context of the CDSS - enabling the description of distinctive features of blood cell morphology in neonates with DS. However, the future of morphology merits further discussion. While it may be very interesting, it is very time consuming, and the role of newer technologies such as the CellaVision DM automated microscope should be explored. If these are able to provide at
least as much information as manual microscopy yields, and if they can do so accurately and reliably, but in a more timely manner, then this would be a considerable advantage over manual review alone.

10.3 Ongoing and future work

There is considerable interest in TMD, and as discussed, there is uncertainty about basic questions such as the incidence of GATA1s mutations at birth. Further work is now underway to extract DNA from the neonatal samples to determine the incidence of GATA1s mutations in the CDSS population. This can then be correlated with the automated and manual analysis already completed on these samples. It is already clear that blasts are a frequent finding in blood films from DS neonates and the haematological parameters of a single full blood count in a child with TMD that is already regressing may be unremarkable. This suggests that GATA1 mutation analysis may be required if all cases of TMD are to be identified. GATA1 mutation analysis is costly, time-consuming and not freely accessible – a national strategy is therefore required. This work will inform that discussion by demonstrating whether or not automated and manual analysis was sufficient in identifying those with TMD.

One of the strengths of the CDSS is the potential for correlation with clinical data. Once the obstetric and neonatal abstraction is completed it will be possible to correlate the neonatal results with the clinical findings. This will enable specific questions to be addressed in the future, including:

1. Did any of the neonates receive treatment for polycythaemia?
2. Can algorithms currently in use for the management of suspected neonatal sepsis which include the neutrophil count be validated in DS?
3. Did any of the neonates have bleeding problems?
4. Did any of the neonates receive platelet transfusions?
5. Do maternal obstetric factors have any effect on the haematology parameters?

It will also be possible to look at the incidence and associations with other congenital abnormalities associated with DS. Later on it will also be possible to obtain clinical data from older children in the cohort, which would enable further questions to addressed, including:

1. What is the clinical significance of neonatal polycythaemia beyond the neonatal period? In particular, is there any effect of neonatal polycythaemia on subsequent
development, neurological sequelae or the development of secondary polycythaemia in children with congenital cyanotic cardiac disease?

2. What is the clinical significance of leucopenia?

3. Can algorithms for the management of suspected neonatal sepsis which include the neutrophil count be validated in DS?

4. What is the pattern of infectious episodes throughout the early years? Is it possible to identify those most at risk of serious or frequent infection? Is there a relationship between the pattern of infections and leucopenia?

Although the initial impetus for the CDSS came from a desire to study the haematology of children with DS, its scope rapidly enlarged to include all aspects of health with the aim of developing a cohort that also would be useful to the wider medical community. At present three collaborations are nested within the study:

1. Investigating the prevalence of autism spectrum disorder in children with Down syndrome;
2. Examining early communication skills in children with Down syndrome;

Further information about these is found in the study protocol (Appendix 11).

There are many other questions thrown up by this research which lie outside the current scope of the CDSS. In particular, these include:

1. Given the frequent hypogranularity of neutrophils, are there any differences in neutrophil function in DS?
2. Given the frequent pallor of platelets, are there any differences in platelet function in DS?
3. What are the reference haematology values for a cohort of UK neonates?
4. What are the morphological appearances of blood cells in healthy UK neonates – when reviewed in the manner described here?
10.4 Overall conclusion

The research supports the hypothesis that neonates with DS have a distinct haematological profile, and this has been characterised in what is the largest report of DS neonates to date.

The pattern of these changes is consistent with chronic intrauterine hypoxia.

For the first time factors affecting the neonatal full blood count in DS neonates have been examined, and the morphological appearances of the blood films in this population have been described.

It has been demonstrated that automated analysis is insufficient to identify TMD, and manual assessment of the blood cell morphology is indicated in all neonates with DS.

Importantly, it has also been shown that the children in the CDSS continue to have a distinct haematological profile throughout their early years – and this will need to be confirmed in other series.
Appendices

Appendix 1. Calculation of red blood cell indices.

1. HB is determined by measuring the optical density of a solution of haemoglobin or a derivative, such as cyanmethaemoglobin, at a particular wavelength. HB is expressed as g/dl (mass concentration) or as mmol/l (molar concentration).

2. PCV is defined as the proportion of a column of centrifuged blood that is occupied by RBC expressed as a decimal fraction indicating the l/l amount. PCV was initially synonymous with the haematocrit (HCT). However, the term PCV is now reserved for values obtained using manual techniques, whilst haematocrit (HCT) indicates a value determined using an automated method. Modern analysers may measure a microhaematocrit using centrifugation, although this is affected by many factors such as duration and force of centrifugation, size of capillary tube, amount of anti-coagulant and presence of red cell changes like spherocytosis or sickle cell disease. Alternatively, they may determine the HCT by measuring the number and size of RBC passing through a sensor deriving the HCT using the formula:

\[
HCT = \frac{RCC \times 10^{12}/l \times MCV (fl)}{100}
\]

3. RCC is measured directly using an electrical impedance method: as individual blood cells pass through a narrow aperture they temporarily impede the flow of current between two electrodes. Each interruption corresponds to an individual cell. Although, white blood cells (WBC) will be included in the count this is considered acceptable as they are usually present 100-fold less often than RBC. RBC are expressed x 1012/l.

4. MCV is a measure of the average size of a circulating RBC. It may be measured directly using laser optics or an aperture-impedance method as the magnitude of the drop reflects the volume of the cell, expressed in femtolitres (fl). Alternatively, it may be calculated using the RCC and HCT, although the HCT should be corrected to account for the plasma trapped between the RBC to avoid over-estimating the MCV:

\[
MCV (fl) = \frac{HCT \times 1000}{RCC \times (1012/l)}
\]
5. The MCHC is a measure of the HB concentration in an average circulating RBC. Most modern analysers measure the HB and use a calculated value for the HCT to give the MCHC using the following:

\[
\text{MCHC (g/dl) = } \frac{\text{HB (g/dl)}}{\text{HCT}}
\]

6. The MCH is a measure of the average amount of HB in a circulating RBC. This may be calculated using directly measured values for the HB and RCC:

\[
\text{MCH (pg) = } \frac{\text{HB (g/dl)}}{\text{RCC (x 1012/l)}}
\]

7. Red blood cell distribution width (RDW) is a quantitative measure of variation in RBC size, expressed as either the standard deviation (SD) or the coefficient of variation (CV) of individual measurements of RBC volume;

8. Nucleated red blood cells (NRBC), being nucleated, are often mistakenly included in the automated white blood cell count (WBC). More sophisticated analysers are now able to differentiate NRBC from WBC using cluster analysis based on differences in fluorescence intensity and forward light scatter. This enables them to be counted (so that an accurate WBC can also be calculated by subtracting the NRBC from the total nucleated cell count (TNCC).

9. Reticulocytes are young RBC recently released from the bone marrow, and still containing ribosomal RNA which may be directly stained or labelled with fluorochromes. Reticulocytes may then be detected by light scatter or light absorbance and are expressed as either an absolute count or as a percentage of total RCC. The amount of RNA required for a reticulocyte to be counted varies from machine to machine and so reference ranges are specific to each instrument type.
Appendix 2. United Kingdom Accreditation Service and Clinical Pathology Accreditation Ltd.

Within the UK there are two laboratory accreditation bodies, operating in complementary fields: the United Kingdom Accreditation Service (UKAS) and Clinical Pathology Accreditation UK Ltd (CPA). These operate as a partnership which enables the two organisations to co-operate on the development of accreditation policy and facilitates the exchange of best practice. The partnership is aimed at strengthening the authority and reputation of accreditation both in the UK and internationally by bringing together two organisations with established reputations in their respective fields. It is also a means of reducing the risk of fragmenting accreditation and avoiding proliferation of accreditation standards for laboratories. UKAS and CPA also work together to develop international recognition of accreditation. UKAS is recognised by Government as the national accreditation body and is the signatory of international mutual recognition agreements on behalf of the UK. It is intended that the partnership between UKAS and CPA will, in due course, be incorporated into these agreements.

The partnership is the culmination of discussions, which started with a joint statement of intent in 1998 to co-operate in areas of mutual interest and benefit. The introduction of international standards for laboratory accreditation [ISO 17025 & ISO 15189] set a benchmark so that UKAS and CPA could agree on common criteria making a partnership possible as well as desirable to prevent duplication. At present, each organisation aims to maintain their corporate identity and to retain respective control over professional [technical] decisions and standards. A Council, set up to oversee the development of the partnership, meets regularly to discuss the development and activities of the partnership. A number of policies have been agreed to facilitate the partnership, including exchange of information, confidentiality, and competition. Details of these can be found on the websites together with more information on both organisations: www.ukas.com and www.cpa-uk.co.uk.
Appendix 3. UK National External Quality Assessment Scheme for Haematology.

The aim of the UK National External Quality Assessment Scheme for Haematology is to help to improve and maintain performance of laboratory haematology at a high level of proficiency. External quality assessment for haematology in the UK was first introduced in 1968 by the British Committee for Standardization in Haematology (BCSH). The project was financed by a modest grant from the Nuffield Provincial Hospitals Trust. The results of the early trials were reported by S M Lewis and B J Burgess in the British Medical Journal, 1969, 4:253-256. From this developed the UK National External Quality Assessment Scheme for Haematology (UK NEQAS(H)) which currently has about 650 participants. This scheme is concerned with general haematology. There are also complementary national schemes for blood coagulation testing, blood transfusion laboratory practice, haematinic assays, leucocyte immunophenotyping and feto-maternal haemorrhage.

Two specimens are used for analysis of the full blood count. These whole blood specimens are pooled human donations (collected into CPD-A1 anticoagulant) and partially fixed. Blood is dispensed into vials aseptically, using a special mixing-dispenser unit to ensure identical aliquots. The shelf life of the partially fixed human product is at least four weeks at 4°C. Red cell concentration may be varied by removal or addition of plasma. White cell and platelet concentration may be varied by removal or addition of cells (using appropriate filters and/or centrifugation) or by addition of buffy coat and/or platelet concentrates.

Full blood count surveys are distributed 12 times a year.

The lack of internationally recognised standards, except for haemoglobin, necessitates the use of a consensus target value to establish acceptable limits of performance. As the many different analysers available use a variety of technologies and diluents, they may respond in different ways to the stabilised material used in UK NEQAS (H) surveys. Performance is therefore assessed within instrument groups against the consensus target value.

Further information may be found on the UK NEQAS websites: www.ukneqas.org.uk and www.ukneqas-haem.org.uk.
Appendix 4. Study protocol

Protocol for the study of health of babies and children with Down’s syndrome

Identification, recruitment and follow-up
i. Details of initial recruitment
ii. Subsequent follow-up
iii. Contact with the families after consent has been received
iv. Collection of information about clinical status
v. Collection of the biological samples

Deviations from usual process
i. Other routes of entry into the study
ii. Adopted children with Down’s syndrome

Research Plan
i. Study area
ii. Investigating the prevalence of autism spectrum disorder in children with Down syndrome
iii. Examining early communication skills in children with Down syndrome
iv. Investigating the relationship between child’s health and development in children with Down syndrome
v. Investigating early cognitive development in children with Down syndrome
vi. Sample management
   a) Analysis of blood samples
   b) Analysis of buccal swabs
vii. Data management
viii. Communication and dissemination of results

Appendices
Appendix 1: flow chart describing initial recruitment
Appendix 2: flow chart describing contact after hospital entry
Appendix 3: flow chart describing follow-up
Appendix 4: membership of the steering committee
Appendix 5: figure to show Regional Neonatal Networks
Appendix 6: list of hospitals within each Neonatal Network Area
Appendix 7: proforma for review of blood cell morphology
Appendix 8: triggers for referral to a Paediatric Haematologist
Appendix 9: study newsletters

Version 5 25th November 2010
Identification, recruitment and follow up

i. Details of initial recruitment

Recruitment into the newborn stage will usually occur in the hospital setting. This study will therefore involve multiple hospitals across a wide geographical area. Most children born with DS are first diagnosed after birth and will be managed initially by a Consultant Paediatrician, often a Consultant Neonatologist. In order to set up the study, we have approached the Paediatricians or Neonatologists as appropriate in each hospital. Each participating hospital will then designate a named clinician, usually a senior member of the team which normally looks after newborns with DS, who will act as a local contact for the study. In most cases this will be a Consultant Paediatrician or Neonatologist. This named clinician will be responsible for updating their colleagues about the study.

Identification of cases, approach and recruitment into the newborn stage will take place after birth and prior to hospital discharge. When a newborn is thought to have DS a member of the clinical team managing the care is requested to send a card, provided in the newborn pack supplied to each participating hospital, to the Epidemiology & Genetics Unit (EGU) to alert us to a potential new study entrant at as early a stage as possible. This card will be sealed and will only contain the child’s gender, date of birth and notifying hospital. This is a means to improve communication and recruitment rates.

Once the diagnosis is confirmed the study will be discussed with the family by a member of the clinical team managing the newborn’s care as part of discussions normally held at this time.

- Parent Information Sheet
- Parent consent form - newborn stage.

Consent will be sought:

- to do further tests on a full blood count sample. In most cases this will not require an extra sample of blood – we will simply be able to retrieve an existing sample from the appropriate laboratory.
- to allow us to approach the family again to discuss the next stage of the study.
- To access the mother’s maternity records

It will be possible for parents to give permission to any or all of these requests, and it will be possible for them to withdraw their baby from the study at any point if they change their mind. The consent form will be provided in triplicate so that one copy can be kept by parents, one copy can be filed in the notes, and one copy will be sent to us in a prepaid, addressed envelope.

In the majority of cases a blood sample will already have been taken for a full blood count, and this will be suitable for the study. We will retrieve the sample once a family has given consent. In the minority of cases where a full blood count has not been sent already, this will be taken if consent to do so is given by the family. This will then be sent to the Haematology Laboratory at York Hospital for analysis. The results of this will be fed back to the local clinician. The sample will then be collected for study purposes.

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A flow chart describing this process is provided in Appendix 1.

ii. Subsequent follow up

Parents will have a copy of the consent form and also the information sheet which includes information about how to contact the study team if they have any subsequent queries relating to the study. To make this as accessible as possible we have provided a free phone number, an email and postal address as well as a website.

Once babies with DS have been discharged home they will come under the care of their local family practitioner and will also be followed up by a Consultant Paediatrician. We will contact these professionals to inform them that the baby has been entered into the study, and will provide them with information about this:

- Letter to GP/Health Visitor
- Letter to hospital contact
- Information Sheet for Health Professionals

iii. Contact with the families after consent has been received

When we have received the consent form from the newborn stage, we will write to those families whose child is still alive and who have given us permission to contact them. At this point parents will be provided with further information about the study and will be invited to let us know when they are ready to take part in the next stage of the study. They will do this by returning a sealed, pre-paid response card with their current contact details on. This can be returned at any time, allowing families to participate in the study at their own pace:

- Letter to parents after newborn consent
- Family response card

Upon receipt of the response card, families will be sent a study pack inviting them to consent to further follow up. Parents will be asked to sign and return a consent form. A pre-paid addressed envelope will be provided, and a telephone number given so that any queries can be discussed. Consent will be requested for:

- Completion of questionnaires at home;
- Allowing access to the medical records of the child;
- Allowing an additional blood sample to be taken when the child is having additional blood tests and at approximately yearly intervals;
- Allowing a mouth/buccal swab to be taken from the child and both biological parents, where this is possible.

If families have not contacted us or returned the response card by the time their child is 4 months old, we will write to the parents again to invite them to follow up. A flow chart describing the process of subsequent follow up and contact is provided in Appendices 2 & 3.
iv. Collection of information

In summary, information about the child’s health will be collected from:

- parental questionnaires;
- parental interviews
- obstetric, neonatal and paediatric hospital records;
- local family practitioner and health visitor records;
- the Personal Child Health Record (the Red Book).

A number of questionnaires will be sent to parents at differing time points in the study and parental interviews will also be conducted. Details are outlined below.

a) The family questionnaire

The family questionnaire (Family questionnaire ver01 12 May 2009) will be sent in the study pack. The main purpose of the questionnaire is to check family and health care contact details as well as gathering information about the family and reproductive history.

b) Health and Development questionnaires

Parents will be invited to complete a short questionnaire (Health questionnaire 1st ver01 & Health questionnaire FU ver01) about their child’s health and development which is based on the special child development sheet for children with Down syndrome found in the child’s personal health record. The questionnaire will take between 5-10 minutes to complete and will be sent to families at each birthday. Prepaid envelopes will also be provided to the families for return of questionnaires. Families who do not return questionnaires within a month will be contacted again by telephone by trained CDSS research staff. The covering letter which accompanies the questionnaires will include the research teams contact details and parents can contact the team as necessary for advice (Letter to parents health questionnaire).

c) Communication skills questionnaires

Parents will be invited to complete three questionnaires (background questionnaire ver01, language questionnaire ver01, communication questionnaire ver01) to examine early motor, language and communication skills in children with Down syndrome. The questionnaires, which will take between 10-20 minutes to complete, will be sent to families within three months of the child’s 3rd birthday. Prepaid envelopes will also be provided to the families for return of questionnaires. Families who do not return questionnaires within a month will be contacted again by telephone by trained CDSS research staff. The covering letter which accompanies the questionnaires will include the research teams contact details and parents can contact the team as necessary for advice (Letter to parents communication skills questionnaires).
d) **Autism screening parental questionnaires**

Parents will be invited to partake in the completion of two questionnaires to look at the prevalence of autism spectrum disorder in children with Down syndrome each of which will take approximately 5 minutes to complete. Within one month following each child’s 3rd, 4th and 5th birthdays, families will be sent two autism screening parental questionnaires: the social communication questionnaire (SCQ) and the M-CHAT. Families will be asked to return the questionnaires in prepaid envelopes. If questionnaires are not returned **within a month**, trained CDSS research staff will contact the family by telephone. Families who then do not return the questionnaire will not be contacted again until after their child’s subsequent birthday, when questionnaires will again be sent. The covering letter which accompanies the questionnaires will include the research teams contact details and parents can contact the team as necessary for advice (Letter to parents autism questionnaire).

e) **Early cognitive development interviews and questionnaires**

In order to gather information about cognitive development parents will be invited to take part in a series of interviews over a 2 year period which will start when their child is between 4 and 5 years of age. These interviews are likely to take place at home but they can be held at the child’s school or nursery or the families can come to the University of York. There will be three assessment points (T1 – 4 years 6 months, T2 – 5 years 4 months, T3 – 6 years 2 months) between the ages of approximately 4.5 and 6.5 years in which the development of cognitive and language skills will be measured using standardised cognitive tests and experimental tasks. There will be baseline cognitive measures repeated at every time point and additional measures at T2 to enable comparisons to be made with existing data from children with specific language impairment (SLI). Normally each assessment will be completed in a single session but some families may need to be revisited to ensure cooperation of the participating children. One of the tasks will be video recorded; a 10-minute play session between the experimenter and child which will give a sample of the child’s natural language and other language tasks may require audio recordings. Consent for both video and audio recordings will be requested from the parents of participating children (see Consent form Early Cognitive Development; Version 1: 22nd November 2010). All measures will be presented to the children as games and involve pictures, puppets and/or toys to make them fun. Questionnaires (General Health Questionnaire, SWAN, Vineland II) will be completed by the primary caregiver at T1 and T3 during the child assessment if done at home and background information will also be obtained at this time (Family interview Early Cognitive Development; Version 1: 22nd November 2010). Otherwise, they will be sent to parents with a request to return them in pre-paid envelopes to the CDSS group at the University of York. The semi-structured interviews will be conducted face-to-face where possible but via phone if necessary.

Parents will be provided with an information sheet (Parent information leaflet Early Cognitive Development: version 1; 22nd November 2010) about the proposed research as well as the consent form (Consent form Early Cognitive Development; Version 1: 22nd November 2010) and the covering letter (Letter to parents Early Cognitive Development;

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1 It is easier to transcribe from video than audio recordings given articulation problems that are frequently associated with Down syndrome.
Version 1: 22nd November 2010) which includes the research teams contact details and parents can contact the team as necessary for advice. If they parents decide to consent to take part in the study they are asked to provide the best times to be contacted.

v. Collection of the biological samples

Parents of children with DS have been found in previous studies to be a particularly highly motivated group with regard to the management of their child. After consultation with families of a child with DS and after discussion with members of the DSMIG at a National Meeting we have designed the sample collection to reflect this so that families are empowered as partners in the study. The degree to which they wish to take responsibility will vary from family to family, and will be negotiated in each case in discussion with a member of the research team.

Mouth/buccal swab kits are like small toothbrushes which are brushed around the inside of the mouth and in doing so collect cells from which DNA can be extracted. The procedure is non-invasive, painless and straightforward and EGU has considerable experience in using this method for obtaining DNA. Once the procedure is completed the swab needs to be placed inside a container and returned to EGU. The packs for taking the swabs, which include instructions and a pre-paid addressed envelope, will be sent directly to the families as part of the study pack described above. Families will be offered the choice of taking the swabs themselves, or of having them done by a health professional.

Blood samples will be taken when the child is having routine blood tests. The aim is to obtain samples at approximately yearly intervals. In most cases yearly tests of thyroid function are taken in children with DS, in line with DSMIG guidelines. However, we appreciate that practice may vary. If the doctor managing the care of the child in our study is doing tests at a different time then we will modify our process accordingly.

Again, in order to empower the families, the kits for blood sample collection will be sent to them directly. They will then take these packs to the clinic when a blood test is due. The pack contains an EDTA tube, and a pre-paid addressed envelope for return to the Haematology Laboratory at York Hospital, where a full blood count and slide analysis will be performed. The remaining sample will be transferred to the EGU for further analysis and storage. Once a sample has been returned to EGU, a new blood sample collection kit will be sent out to the family.

A flow chart describing the process of follow up is provided in Appendix 3.

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Deviations from usual process

i. Other routes into the study

In many places there is a close knit community of families who have a child with DS which may be a geographical community where families live near one another and meet up as they access similar resources, or, increasingly this may be a community which, although its members are geographically distant, communicates frequently using the internet. Information about the study is accessible on the internet. The study website (www.cdss.org.uk) includes a message board for parents along with information about the study. There are links to this website on both the DSMIG and DSA websites.

The DSA has been involved in setting up this investigation and information about the study is routinely included in the journal it produces. It is possible that as a result of this we may be approached directly by parents who are keen to participate in the study. It is also possible that families who have initially declined to enter the study might change their minds, and might subsequently wish to join. In all cases the families will be welcome to join.

ii. Adopted children with Down’s syndrome

Babies with DS are more likely to be offered up for adoption than are other babies. It is possible that a baby who has been entered into the study as a neonate may be subsequently offered up for adoption. In this case we would not be able to follow the child up further. Moreover, as we will always contact the general practitioner or health visitor before we make contact with a family prior to follow up we would learn of this and so would not go on to contact the family.

It is also possible that a family who have adopted a baby or child with DS may hear about the study and may wish their child to join the study. Provided that they are eligible they would be able to do so as a late entrant following the process outlined above. There is a modified entry questionnaire to be used in this scenario.
Research Plan

The study is being overseen by a steering committee that meets annually. Details of the membership, which includes treating clinicians and the DSA, are provided in Appendix 4.

i) Study area

Established Regional Neonatal Networks form the geographical basis of the study (Appendix 5). Initially it will be piloted in the area covered by the Yorkshire Neonatal Network. It will then be rolled out to include two adjacent Neonatal Networks during 2006, and two more Neonatal Networks will be recruited in 2007. Details of the current hospitals within each of these Networks are provided in Appendix 6.

Other Neonatal Networks will join the study as it develops.

ii) Investigating the prevalence of autism spectrum disorder in children with Down Syndrome

Families who are enrolled in the study and who have consented to be contacted again will be sent within one month following each child’s 3rd, 4th and 5th birthdays two autism screening parental questionnaires: the social communication questionnaire (SCQ) (Berument et al 1999) and the M-CHAT (Robins et al 2001). Families will be asked to return the questionnaires in pre-paid envelopes. If questionnaires are not returned within one month, trained CDSS research staff will contact the family by telephone. Families who then do not return the questionnaire will not be contacted again until after their child’s subsequent birthday, when questionnaires will again be sent.

After age 5 years, questionnaire total scores will be compared with the child’s clinical diagnosis from their medical case notes. The sensitivity and specificity of the questionnaire scores for the clinical diagnosis of ASD in children with DS will then be calculated.

Findings from the questionnaires will not be given to parents directly, as clinical relevance of the scores cannot be interpreted without clinical assessment of the child. Results will be shared with the child’s paediatrician, with parental consent, so they can be considered in a clinical context. When children are not under paediatric care, results will be shared with GPs. When a child has high SCQ and/or M-CHAT scores, the standard letter will indicate that the child has potentially significant social communication difficulties, which warrant assessment by a paediatrician.

Initially, families will annually receive two questionnaires about ASD behaviours which will each take approximately 5 minutes to complete. We consider that whilst this is an additional aspect to the families involvement in the core research study, the burden for families (frequency or duration) is acceptable.

It is possible that through the completion of these questionnaires, that parents might become concerned that their child has autism or ASD. The covering letter which accompanies the questionnaires will include the research teams contact details and parents can contact the team as necessary for advice (Letter to parents autism questionnaires). When this occurs, the

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research team will suggest that the family contact their local paediatrician. When high SCQ and/or M-CHAT scores are recorded, this potentially clinically important clinical information will be communicated in a letter to the paediatrician and/or GP, and a paediatric assessment suggested (Letter to GP after ASD questionnaires).

**iii) Examining early communication skills in children with Down syndrome**

Families who are enrolled in the study and who have consented to be contacted again will be sent three questionnaires to complete within three months of their child’s 3rd birthday (background questionnaire ver01, language questionnaire ver01, and communication questionnaire ver01) to examine early motor, language and communication skills. The questionnaires will take between 10-20 minutes to complete. Families will be asked to return the questionnaires in pre-paid envelopes and if the questionnaires are not returned within a month, trained CDSS research staff will contact the family by telephone.

Using the information we collect from the questionnaires we plan to investigate

- **The relationship between motor skills and preverbal**
  - Are there early difficulties in preverbal social communication and are these linked to motor difficulties?
  - Do children with Down syndrome use alternative methods of preverbal social communication?

- **The relationship between preverbal communication and later verbal skills**
  - Do those children who have better preverbal skills go on to develop better verbal skills?

- **The relationship between signing and speaking**
  - Is learning sign language beneficial to spoken language development in children who have Down syndrome?

Findings from the questionnaires will not be given to parents directly, as they do not provide information that is not already known to the parents and it is not possible to provide meaningful scores because the questionnaires are not “normed”. However, a summary of the overall findings will be sent to participating families at the end of the study.

The covering letter which accompanies the questionnaires will include the research teams contact details and parents can contact the team as necessary if they have any questions or require additional information (Letter to parents communication skills questionnaires).
iv) Examining the Health and Development of children with Down Syndrome

Families who are enrolled in the study and who have consented to be contacted again will be sent a questionnaire to complete around the time of their child’s birthday (Health questionnaire 1st ver01 & Health questionnaire FU ver01). The questionnaire will provide the opportunity to check that we have the correct family and health care contact details for the child as well as asking a few questions about their child’s health and development. The questions we are asking are based on the special child development sheet for children with Down syndrome found in the child’s personal health record and will take between 5-10 minutes to complete.

Families will be asked to return the questionnaires in the prepaid envelopes provided. If families do not return questionnaires within a month weeks they will be contacted again by telephone by trained CDSS research staff.

Findings from the questionnaires will not be given to parents directly, as they do not provide information that is not already known to the parents. However, a summary of the overall findings will be sent to participating families at the end of the study.

The covering letter which accompanies the questionnaires will include the research teams contact details and parents can contact the team as necessary for additional information or if they have any questions (Letter to parents health questionnaire).

v) Investigating early cognitive development in children with Down syndrome

Families who are enrolled in the study and who have consented to be contacted again will be sent an information sheet (Parent information leaflet Early Cognitive Development: version 1; 22nd November 2010) about the proposed research as well as the consent form (Consent form Early Cognitive Development; Version 1: 22nd November 2010) and the covering letter (Letter to parents Early Cognitive Development; Version 1: 22nd November 2010) which includes the research teams contact details and parents can contact the team as necessary for advice.

The families will be invited to take part in a series of interviews that can be held in their own home, at their child’s school or nursery or at the University of York. The first of these will most likely be conducted at the child’s home so as not to inconvenience the family with subsequent sessions held at whichever location the family decide most suitable. These interviews will take place over a 2 year period at three time points, 4 years 6 months (T1), 5 years 4 months (T2) and 6 years 2 months(T3) and are likely to take 1-2 hours. At these time points the development of cognitive and language skills will be measured using standardised cognitive tests and experimental tasks (see below for more detail relating to tests and tasks). One of the tasks will be video recorded; a 10-minute play session between the experimenter and child which will give a sample of the child’s natural language and other language tasks may require audio recordings. All

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1 It is easier to transcribe from video than audio recordings given articulation problems that are frequently associated with Down syndrome.

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measures will be presented to the children as games and involve pictures, puppets and/or toys to make them fun.

At the first and last time-points, there are questionnaires and interviews for the primary caregiver (see below for more details). The questionnaires should take no more than 1 hour to complete and can be done during the child assessment and the interviews will last a similar length of time and can be done before or after the child assessment.

Tests and tasks

**Time point 1 (T1) – 4.5 years old**

### Language skills

<table>
<thead>
<tr>
<th>Test</th>
<th>Task</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expressive language</td>
<td>Video natural language sample of child’s speech during a 5 minute play session used to calculate mean length of utterance (MLU)</td>
<td></td>
</tr>
<tr>
<td>Phonological memory</td>
<td>child repeats words and novel words after the examiner</td>
<td>Roy P, Chiat S &amp; Seiff-Gabriel. (2008). <em>Early Repetition Battery (ERB)</em>. Pearson. (PSRep subtests) -</td>
</tr>
</tbody>
</table>

### Nonverbal and motor skills

<table>
<thead>
<tr>
<th>Test</th>
<th>Task</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visualisation &amp; Reasoning</td>
<td>child assembles fragmented puzzle pieces and completes incomplete repeated patterns respectively</td>
<td>Roid GM, Miller L.J. (1997). <em>Leiter International Performance Scale-Revised (Leiter-R)</em>. Stoelting Co. (Form completion and repeated patterns subtests)</td>
</tr>
<tr>
<td>Visual memory</td>
<td>child points to a sequence of visual images in the same order as the examiner</td>
<td>Roid GM, Miller L.J. (1997). <em>Leiter International Performance Scale-Revised (Leiter-R)</em>. Stoelting Co. (Forward memory subtest)</td>
</tr>
<tr>
<td>Test</td>
<td>Task</td>
<td>Reference</td>
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<td>-----------------------------</td>
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</table>

*Time point 2 (T2) – 5 years 4 months old*
|----------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| Phonological awareness           | child selects picture from a choice of two that starts or ends with the same sound as a given word | Carroll JM, Snowling MJ, Stevenson J & Hulme C. (2003). The development of phonological awareness in preschool children. *Developmental Psychology*, 39, 913-923. (syllable)  

**Nonverbal and motor skills**

<table>
<thead>
<tr>
<th>Test</th>
<th>Task</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual memory</td>
<td>child points to a sequence of blocks, that increase in length, in the same order as the examiner.</td>
<td>Pickering, S. &amp; Gathercole, S. (2001). <em>Working Memory Test Battery for Children (WMTB-C)</em>. The Psychological Corporation (block recall subtest)</td>
</tr>
<tr>
<td>Hearing threshold</td>
<td>Audiometry</td>
<td></td>
</tr>
</tbody>
</table>

*time point 3 (T3) 6 years 2 months--*

This time point is the second follow-up of the DS sample. All measures are the same as those given at T1 and the questionnaires and interviews are repeated with parents.
Questionnaires and interviews

Questionnaires (i) General Health Questionnaire, (ii) SWAN, (iii) Vineland II will be completed by the primary caregiver at T1 and T3 during the child assessment if done at home and background information (iv) will also be obtained at this time (Family interview Early Cognitive Development; Version 1: 22nd November 2010). Otherwise, they will be sent to parents with a request to return them in pre-paid envelopes to the CDSS group at the University of York. The semi-structured interviews will be conducted face-to-face where possible but via phone if necessary. Normally each assessment will be completed in a single session but some families may need to be revisited to ensure cooperation of the participating children.

(i) General Health Questionnaire (Goldberg D. (1992). General Health Questionnaire (GHQ-12). NFER-Nelson) is a self-report questionnaire to assess feelings of stress and dejection in the primary caregiver so that this can be examined as a potential correlate of cognitive skills.

(ii) SWAN (Swanson et al., (2006). Categorical and dimensional definitions and evaluations of symptoms of ADHD: the SNAP and SWAN rating scales. University of California, Irvine.) is a published questionnaire that assesses attention in young children so that this can be examined as a potential correlate of cognitive skills.


(iv) The semi-structured interview (Family interview Early Cognitive Development; Version 1: 22nd November 2010) will enable information to be obtained on child’s health issues, life events and family stresses, family structure, family literacy and socio-economic background as these could all be important correlates of individual differences in cognitive skills.

Using the information obtained we aim to

- To describe the early cognitive phenotype of children with DS
- To examine if the documented dissociation between language and non-verbal skills in DS is evident at this young age.
- To examine the role of a wide range of health and social factors in determining variability in cognitive skills.
- To examine whether children with DS have a comparable language profile to those with specific language impairment (SLI) using existing data.
The results of standardised tests will be fed back to parents. Members of the research team will be available by telephone to clarify these results and to provide support but will not give recommendations for intervention since these are beyond the remit of the project. Families with concerns will be encouraged to share the reports with relevant professionals and advice will be given when appropriate as to how to seek help and support.

vi) Sample management

a) Analysis of blood samples

The neonatal blood sample will be analysed as follows:

- Routine full blood count analysis will usually be performed by York Hospital haematology laboratory. This will provide data on the number, size and quality of red blood cells, white blood cells, and platelets and will be fed back to the local contact or the senior clinician managing the newborn’s care.
- Up to three slides will be made for blood cell morphology and will be reviewed by a panel of experienced Paediatric Haematologists using a proforma (Appendix 7);

Subsequent blood samples will be obtained when additional tests are being performed, and sent directly to York Hospital for a full blood count. This will allow standard ranges for haematological parameters in children with DS at different ages to be determined, and will also allow the natural history of abnormalities of the full blood count to be documented. These results will not be routinely fed back either to the local contact or the family. However, if these results are suggestive of a problem that may require clinical management then the result will be discussed with a paediatric haematologist at the Regional Centre for Paediatric Haematology at St James’ hospital in Leeds. In order to standardise the procedure for referral a list of triggers has been drawn up (Appendix 8).

Following processing at York Hospital samples will be transferred to EGU where DNA and RNA will be extracted. Further analysis will be carried out at the most appropriate facility in the UK with the necessary expertise. Where samples are sent to laboratories other than the Haematology Laboratory at York Hospital and EGU for testing they will be fully anonymised.

b) Analysis of buccal swabs

Buccal swabs will be sent directly to EGU. DNA and RNA will be extracted for constitutional genetic and gene expression studies which will be carried out at facilities with the most appropriate expertise. Samples will be fully anonymised prior to sending to other laboratories.
vi) Data management

All children enrolled in the study are given a unique identifier. Data and information collected throughout the study along with results of sample analysis will be held in a secure database. All questionnaires and additional paperwork will be stored appropriately in locked cabinets for future use.

vii) Communication and dissemination of results

Regular progress reports are produced and the first two newsletters are provided in Appendix 9. In addition, results of analyses will be disseminated through:

- publication in peer-reviewed journals;
- presentation at medical and scientific meetings including those of the Regional Neonatal Networks involved and of the DSMIG;
- the study newsletter and website;
- the journal of the DSA.
Appendix 5. DSMIG schedule for health checks in children with DS.

### Table: Suggested Schedule of Health Checks

<table>
<thead>
<tr>
<th>Appendix 5: DSMIG schedule for health checks in children with DS.</th>
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<tbody>
<tr>
<td><strong>Thyroid blood tests</strong></td>
</tr>
<tr>
<td>Birth to 6 weeks</td>
</tr>
<tr>
<td>6 - 10 months</td>
</tr>
<tr>
<td>12 months</td>
</tr>
<tr>
<td>18 months to 21/2 years</td>
</tr>
<tr>
<td>3-31/2 years</td>
</tr>
<tr>
<td>4-41/2 years</td>
</tr>
<tr>
<td>Routine Guthrie test</td>
</tr>
<tr>
<td>Thyroid blood tests including antibodies</td>
</tr>
<tr>
<td>Thyroid blood tests including antibodies</td>
</tr>
<tr>
<td>If your area has introduced fingerprint blood tests these should be done every year.</td>
</tr>
</tbody>
</table>

| **Growth monitoring**                                         |
| Length and weight should be checked frequently and plotted on Down's syndrome growth charts. (See page 9 onwards) |
| Head circumference should be checked at each routine medical check. |
| Length and weight should be checked at least annually and plotted on Down's syndrome growth charts. |

| **Eye check**                                                  |
| Visual behaviour. Check for congenital cataract                |
| Visual behaviour. Check for squint                            |
| Visual behaviour. Check for squint                            |
| Orthoptic examination, refraction and ophthalmic examination |
| Visual acuity, refraction and ophthalmic examination          |

| **Hearing check**                                             |
| Neonatal screening, if locally available                      |
| Full audiological review (Hearing, impedance, otoscopy)       |
| Full audiological review (Hearing, impedance, otoscopy)       |
| **Heart check and other advice**                              |
| Echocardiogram 0-6 weeks or chest X Ray & ECG at birth and 6 weeks |
| FROM AGE 5 TO 10 YEARS                                        |
| **Faecal test**                                               |
| Annually                                                      |
| **Hearing**                                                   |
| 2 yearly audiological review (as above)                       |
| **Vision / Orthoptic check**                                  |
| 2 yearly                                                      |
| **Thyroid blood tests**                                       |
| At age 5 years, then either 2 yearly review surveillance or annual fingerprint. |

Detailed recommendations for Medical Surveillance Essentials for children with Down's syndrome are available. For further information contact your local community paediatrician.

Down's syndrome insert © DSMIG 2000
Appendix 6. Paperwork needed for the newborn entry pack.

Each newborn pack contained the following:

i. Alert card
ii. Newborn entry pack cover sheet
iii. Parent information leaflet - newborn
iv. Parent consent form - newborn
v. Information sheet for health professional
vi. Referring hospital form
vii. Haematology laboratory blood form

i. Alert card
ii. Newborn entry pack cover sheet

CDSS
Children with Down’s Syndrome Study

INSTRUCTIONS FOR ENTERING A NEWBORN INTO THE STUDY

Contents of this newborn pack:

- Alert card
- Information leaflets:
  - 1 for the family
  - 1 for health professionals
- Consent form
- Referring hospital form
- Prepaid envelope for returning consent form and referring hospital form to CDSS, University of York
- Form for collecting full blood count sample
- Pink EDTA tube for collection of full blood count sample
- Patient identifier label for the blood sample tube
- Prepaid, padded envelope for sending blood sample to York Hospital

The alert card
As soon as you are aware of a potential study entrant, please complete and return the prepaid alert card.

The consent form
After discussing the study with the family and providing them with an information leaflet, we would be very grateful if you could ask the mother to sign the enclosed consent form and then return the white copy to us in the prepaid envelope provided.

The referring hospital form
Please complete the referring hospital form and return with the white copy of the consent form in the prepaid envelope provided.

Obtaining a blood sample
If a blood sample has already been taken, and is still available in your hospital laboratory, please retrieve it and send it to York Hospital in the enclosed prepaid padded envelope.

If a blood count sample is not available, please take one using the tube provided (assuming the family consents) and send it to York Hospital with the form in the padded envelope.

If you would like more information about the study:
There are information leaflets for both health professionals and parents included in this pack. If you would like further information please contact us (details below).

Freephone: 0800 3280655
Email: cdss@egu.york.ac.uk
Website: www.cdss.org.uk

Newborn entry pack cover sheet; Version 5: 8th May 2007
iii. Parent information leaflet - newborn

Why do you need to look at medical records?
We need accurate information about the health of you and your child.

Will the information I provide be kept confidential?
Yes. All information you provide is confidential and kept in accordance with the Data Protection Act.

No one outside the research team will be able to trace or identify you or your child. The samples and information collected will be used for research purposes only.

Does my child have to take part?
It is up to you to decide whether or not you would like your child to take part. If you take part, you are free to withdraw at any time. Your decision will not affect the standard of care your child receives or the relationship with your child's doctors.

What should I do now?
If you wish to take part, you will be asked to read and sign a consent form. You will be given a copy to keep.

What if I change my mind?
You can join or withdraw from the study at anytime. If you wish to do either of these, please contact us using the contact details below.

Standard NHS indemnity arrangements apply to this research.

For more information please call the freephone number, visit our website or email the address below:

Freephone: 0800 3380655
Email: cdss@eco.york.ac.uk
Website: www.cdss.org.uk

PARENT/GUARDIAN INFORMATION LEAFLET

This is an invitation to take part in a research study. Please take time to read the leaflet carefully and discuss it with other people if you wish. It is important that you understand why the research is being done and what it will involve before you decide whether or not to take part. Please contact us if there is anything that is not clear, or if you would like more information - our contact details are on the back page.

What is the purpose of the study?
Babies and children with Down's syndrome often have more health problems than other children. We aim to find out more about this by collecting as much information about their health as we can.

Who is doing the study?
The study is being done by researchers and doctors and nurses involved in the care of babies and children with Down's syndrome.

Why was my child chosen?
We are interested in all babies and children with Down's syndrome. Some babies may join the study as newborns and other babies and children may join when they are older.

Why should I help?
Information collected about your child could lead to a greater understanding of the health of babies and children with Down's syndrome.

What does the study involve?
The study has several parts but if there is any part you would prefer your child not to be involved in, you can indicate this on the consent form and your wishes will be respected. For babies joining the study as newborns, we would like your permission to have access to a blood sample taken shortly after your baby's birth. In most cases we will be able to use one that has already been taken. If a suitable sample is not available, we would like your permission to take another.

If you agree to participate we will write to you with more information about the next stage of the study. This will include:
- Sending you a short questionnaire before your child's first birthday and then once a year after that.
- Looking at medical records.
- Collecting blood samples from your child when they are having routine tests.
- Collecting buccal (mouth) swabs from you and your child.

Why do you need blood samples from my child?
Children with Down's syndrome are known to be at increased risk of a number of conditions affecting their blood. These may be minor or more serious ones. At present we do not know much about what causes these conditions or about factors influencing their development. By looking at children's blood over several years we hope to find out more.

How will you collect the blood samples?
Most children with Down's syndrome have routine blood tests to monitor their thyroid function. In many regions these are done yearly, though the frequency varies in different parts of the country.

When your child is due to have this blood test, we will send you a small tube to take to the clinic along with instructions.

What happens to my child's blood samples?
These will be stored and will be used for research purposes only.

What happens if you find a serious change in my child's blood sample?
If we see a serious abnormality in a test result that needs to be followed up, we will contact your doctor so that your child can receive the best care possible.

Why do you need buccal (mouth) swabs?
From cheek cells in your mouth we can get DNA which we will be able to store for research.

Parent information leaflet (a): version 2: 13th July 2007
iv. Parent consent form - newborn

CDSS
Children with Down's Syndrome Study

NEWBORN CONSENT FORM

Thank you for reading the information about this study. If you would like to take part, please read and sign this form. Please initial each box if you agree with the accompanying statement:

1. I have read the study information leaflet and have been given a copy to keep. I have been able to ask questions about the study and I understand why the research is being done.

2. I understand that my baby's participation in this study is entirely voluntary and that I will not receive any payment. I am free to withdraw my consent at any time without giving a reason and without my baby's medical treatment being affected.

3. I am aware that my baby may have already given samples for routine purposes. I agree to these samples being stored and used for future research projects.

4. If a new sample of blood is needed from my child, I agree to this being taken. (Your child may still take part in the study even if you do not wish to give permission for a new sample.) I agree to this sample being stored and used for future research projects.

5. I give my permission for a member of the research team to access and record information from my maternity records.

6. I understand that all information about me and my baby will be treated confidentially and that the results from any tests will not be used or released in such a way that we could be identified.

7. I understand that any research project will be approved by the relevant ethics committee.

8. I agree to my child's family doctor (GP) and health visitor being informed that I am taking part in this study.

9. I agree to be contacted again about the next stage of the study.

Mother's surname: ___________________ Mother's first name: ___________________

Signature: _________________________ Date: __/__/___

Baby's surname: ___________________ Baby's first name: ___________________

Address: __________________________

________________________________________

Postcode: ___________________ Telephone number: ___________________

White copy to be returned in envelope to CDSS, PO Box 518, SRB Area 3, York, YO10 5BF; yellow copy to family; green copy for medical records.

Newborn parent consent form: Version 5: 13th July 2007
CDSS
Children with Down’s Syndrome Study

INFORMATION SHEET FOR HEALTH PROFESSIONALS

What is the purpose of the study?
To follow a group of children with Down’s syndrome from birth onwards to investigate their health.

Who is doing the study?
The study is a collaboration between paediatricians and haematologists at St James’ University Hospital in Leeds, and the Epidemiology & Genetics Unit at the University of York. It has been developed with the support of the Down’s Syndrome Association and the Down’s Syndrome Medical Interest Group. We are also working with other health professionals who, like you, are directly involved in the care of children with Down’s syndrome.

When can children be entered into the study?
All babies born from March 2006 onwards are eligible. Most children will be entered into the study as newborns, but older children with Down’s syndrome can also take part in the study.

What will be collected?
For babies joining the study as newborns, we will ask families for consent to have access to a blood sample taken shortly after their baby’s birth. In most cases we will be able to use one that has already been taken. If a suitable sample is not available, we will seek permission to take another. However, if families don’t agree to this it does not prevent them from taking part in the study.

If parents agree to participate in the study we will write to them with more information about what will happen next. This will include sending parents a short questionnaire before their child’s first birthday, and then once a year after that; looking at medical records; collecting blood samples from the child when they are having routine tests and collecting buccal (mouth) swabs from parents and children.

What will happen to the data and samples?
All data will be stored and managed by the Epidemiology & Genetics Unit at the University of York. This information is stored confidentially and kept in accordance with the Data Protection Act.

A full blood count will be carried out on the blood samples. Results from the neonatal samples will be sent to the team managing the baby. If any of the full blood count tests reveal a serious abnormality, advice will be sought from a Paediatric Haematologist at the Regional Centre for Paediatric Haematology in Leeds. The remainder of the samples will be stored and used for research purposes only.

If you want any more information or have any questions about the study, please contact us on:
0800 3280655
cdss@york.ac.uk
www.cdss.org.uk

Information sheet for health professionals; Version 1: 10th May 2007
vi. Referring hospital form

CDSS
Children with Down’s Syndrome Study

REFERRING HOSPITAL FORM

Please complete this form and return with the white copy of the consent form in the envelope provided.

Mother’s name: ________________________________

Mother’s Hospital ID number: ______________________

Baby’s name (if known): _________________________ Baby’s gender: M F

Baby’s date of birth: ___ / ___ / ______

Baby’s hospital ID number: ____________ Baby’s NHS no. (if known): ______________

Gestational age: _____________________________ Birth weight: _____________ grams

Hospital name: ________________________________

GP name: _________________________________

GP address: _________________________________

Is a full blood count sample being sent for the study? Yes [ ] No [ ]

Is this a new sample taken specifically for the study? Yes [ ] No [ ]

If completed, please return the white copy of the consent form, along with this form using the prepaid envelope addressed to:

CDSS
PO Box 518
SRB, Area 3
York, YO1 0BF

If you have any queries please contact us on:

Freephone: 0800 3280655
Email: cdss@eqi.york.ac.uk

255
vii. Haematology laboratory blood form

<table>
<thead>
<tr>
<th>Please complete in capitals/use address label for patient details:</th>
<th>Test required: Full blood count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surname</td>
<td>3 slides – 1 stained</td>
</tr>
<tr>
<td>Forename</td>
<td>2 unstained</td>
</tr>
<tr>
<td>DOB</td>
<td>Save for collection</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Hospital no</td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td></td>
</tr>
<tr>
<td>Requesting Dr</td>
<td></td>
</tr>
<tr>
<td>Venous/capillary</td>
<td></td>
</tr>
<tr>
<td>Date taken</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Please phone 0800 328 0655 if there are any problems, or to let us know about a sample.</td>
</tr>
</tbody>
</table>

Please send 1x 1-2ml in pink EDTA vacuette. PLEASE AGITATE TO PREVENT CLOTTING.
Appendix 7. Standard operating procedure for processing the blood samples in the haematology laboratory.

1 Principle
The Northern Down’s Syndrome Study (www.downs-study-uk.org) is a multi-centre study following the health of children with down’s syndrome from birth to 5 years. Blood samples for the Northern Down’s Syndrome Study will be sent directly from the participating hospital to the haematology laboratory at York hospital by first class post for processing. At time of commencement, up to two children a week are being recruited to the study. This will increase to 2-3 samples in 2007, 2-4 samples in 2008 on current rates. Any requests for changes to the request form below should be made to Dr James (contact details below).

2 References & Contacts
Clinical /Technical contact:
Dr Beki James
Clinical Research Fellow
Epidemiology and Genetics Unit
e-mail: bekjames@egu.york.ac.uk
Tel: 01904 321893
07968 387254

Invoices to be sent to:
Vinnette Sinclair
Epidemiology and Genetics Unit
Department of Health Sciences
University of York
York
YO10 5DD

3 Sample Requirements
1 x 4ml EDTA will be sent.

4 COSHH Risk Assessment

HEALTH & SAFETY
- All human blood samples must be treated as potentially bio-hazardous.
- Identified, Approved Personal Protective Equipment (PPE) must be worn when handling blood samples or derivatives thereof.
- Good Laboratory Practice must be followed at all times.

C.O.S.H.H. RISK ASSESSMENT
The COSHH risks are very low when this SOP is followed in conjunction with the safety precautions outlined.
5 Methods and Results

1. The sample will arrive by First Class post, accompanied with the request form (example shown).

<table>
<thead>
<tr>
<th>The Northern Down’s Syndrome Study</th>
<th>Test required: Full Blood Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 slides - 1 stained</td>
</tr>
<tr>
<td></td>
<td>2 unstained</td>
</tr>
<tr>
<td></td>
<td>Save for collection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Please complete in capitals/use address label for patient details:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Surname</td>
<td></td>
</tr>
<tr>
<td>Forename</td>
<td></td>
</tr>
<tr>
<td>DOB</td>
<td>Sex</td>
</tr>
<tr>
<td>Hospital no</td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td></td>
</tr>
<tr>
<td>Requesting Dr</td>
<td></td>
</tr>
<tr>
<td>Venous/capillary</td>
<td></td>
</tr>
</tbody>
</table>

Please send 1x4ml in pink EDTA vacuette. Please phone 0800 328 0655 if there are any problems, or let us know about a sample.

2. Unpackage and then telephone the Epidemiology and Genetics Unit to let them know the sample has arrived, or if there are any problems with the sample or form. Telephone number is 0800 328 0655. If there is no answer – leave an answerphone message stating your name and contact number. The messages are checked regularly.

3. Book in the sample as part of the Northern Down’s Syndrome Screening Study. (NDSSS).

4. Check sample for clot – report as clotted if clot detected otherwise process for a full blood count with NRBCs and reticulocytes (refer to appropriate SOPs).

5. Make three slides for blood films are label with patient details (refer to appropriate SOPs), staining one and leaving two unfixed, unstained.

6. Authorise FBC result and print out Sysmex parameters, but do not examine any films.

7. Package the sample, and three blood films.

8. Retrieve the hard copy report.

9. Put the sample and films in an appropriate container and place with the Sysmex printout and hard copy report.

10. Label the container for the attention of Buki James, Study co-ordinator, and inform Buki the sample is ready for collection.

11. Take the container down to Ground Floor reception to be collected if the same day, otherwise refrigerate until collection day.
6 Limitations.
This process can only be performed on adequately labeled, uncotted EDTA samples.

7 Invoicing.
Invoices will be generated monthly via Service Level Agreements, and charged at NHS tariffs.

8 Troubleshooting
For all problems contact Buki James (details above).
Appendix 8. Triggers for referral to the Regional Centre for Paediatric Haematology.

List of abnormalities of the full blood count which require discussion with a Paediatric Haematologist at the Regional Centre for Paediatric Haematology

If any of the full blood count parameters are outside the limits below then the results must be discussed with a Paediatric Haematologist at the Regional Centre for Paediatric Haematology at the soonest possible opportunity. In the first instance please contact Sally Kinsey or Beki James.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0-3m</th>
<th>3-6m</th>
<th>6-12m</th>
<th>2y</th>
<th>3y</th>
<th>4y</th>
<th>5y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb g/dl</td>
<td>&lt;7</td>
<td>&lt;7</td>
<td>&lt;7</td>
<td>&lt;7</td>
<td>&lt;7</td>
<td>&lt;7</td>
<td>&lt;7</td>
</tr>
<tr>
<td></td>
<td>&gt;16</td>
<td>&gt;17</td>
<td>&gt;17</td>
<td>&gt;17</td>
<td>&gt;17</td>
<td>&gt;17</td>
<td>&gt;17</td>
</tr>
<tr>
<td>PCV L/L</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td></td>
<td>&gt;0.6</td>
<td>&gt;0.5</td>
<td>&gt;0.5</td>
<td>&gt;0.5</td>
<td>&gt;0.5</td>
<td>&gt;0.5</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>MCV fl</td>
<td>&lt;70</td>
<td>&lt;70</td>
<td>&lt;65</td>
<td>&lt;65</td>
<td>&lt;65</td>
<td>&lt;65</td>
<td>&lt;65</td>
</tr>
<tr>
<td></td>
<td>&gt;110</td>
<td>&gt;110</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>WBC x 10^9/L</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>x 10^9/L</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>x 10^9/L</td>
<td>&gt;12</td>
<td>&gt;12</td>
<td>&gt;12</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Basophils</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>x 10^9/L</td>
<td>&gt;1.5</td>
<td>&gt;1.5</td>
<td>&gt;1.5</td>
<td>&gt;2</td>
<td>&gt;2</td>
<td>&gt;2</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>x 10^9/L</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

If the Paediatric Haematologist believes that further clinical management is required then the study co-ordinator will be informed and the identity of the person from whom the sample was taken will be retrieved. The concerns will then be discussed with the clinician managing that person so that they can take appropriate action. The concerns or results will not be discussed directly between the study team and an individual person.

Version 1 5th February 2006

Standard morphology review procedure

1. Confirm that the identification codes on the slide and on the reporting sheet are the same.
2. Examine the slide under x4 magnification, beginning at the peripheries. Ensure that these have been fully viewed before moving on.
3. Examine the slide under x40 using an oil immersion lens.
4. A white blood cell differential count should then be performed if it is possible to identify >75% of all white blood cells. If there are many disrupted cells then a differential count cannot accurately be performed and should not be attempted.
5. In order to complete the differential white blood cell count 100 consecutive identifiable white blood cells should be counted taking an area of the film where the red blood cells are one cell thick – that is where they do not overlie one another and are approximately adjacent.
6. Pause at each white blood cell to identify its type.
7. Once the type has been identified, consider the appearance of the cell and note whether it has any of the features being considered on the form for that cell type.
8. Once this has been done, move on to the next white blood cell.
9. At the same time as the white blood cell differential is being completed please record the number of nucleated red blood cells seen in order to calculate the nucleated red blood cell count per 100 white blood cells.
10. Once the white blood cell differential has been completed complete the answers to the questions on nuclear/cytoplasmic asynchrony and on blasts.
11. Now, consider the red blood cells. At least twenty fields should be viewed before concluding that the abnormal red cell features being sought are absent.
12. Finally, consider the platelets. Again view at least twenty fields before concluding that the abnormal platelet features being sought are absent.
13. Review the form to check that all the questions have been completed.
Appendix 10. Article about the CDSS published in the quarterly DSA Journal.

Acute leukemia in children with Down’s syndrome: the importance of population based study

The association between Down’s syndrome (DS) and acute leukemia is well documented. However, most information derives from retrospectively compiled patient series or treatment trials, which may not represent the general DS population. We report cases of acute leukemia and DS collected in the UK Childhood Cancer Study (UKCCS), a national population based case-control study. Data were gathered on all children aged 0-14 years diagnosed with acute leukemia between 1991 and 1996, irrespective of trial entry. Detailed diagnostic information on all cases was obtained from multiple sources, including: the Medical Research Council; UKCCSG; the National Registry of Childhood Tumours; the individual treating consultant; and hospital records. In addition, detailed cytogenetic information on trial and non-trial cases was obtained from the Leukaemia Research Cytogenetics Group. Molecular diagnostic information on patients with acute lymphoblastic leukemia (ALL) was provided by the central reference laboratory at the Leukaemia Research Fund Centre, Institute of Cancer Research. Details of the conduct and ethical approval of the UKCCS are described in full elsewhere.

In total, 1,709 children with acute leukemia enrolled in the UKCCS (Table 1). Of these, 1,406 (86%) of the 1,688 with AML and 342 (2%) of the 1,461 with ALL also had DS. Trial uptake was significantly lower for children with DS: 32/48 (67%) children with DS were in trials compared with 1,468/1,709 (86%) of non-DS cases (p<0.001). Entry was lowest for children with DS-AML with only 8/14 (57%) in trials.

We also report the striking finding that the increase in AML incidence in DS was confined to FAB M6 and M7 sub-types; no other FAB types were observed. Eleven (7%) of the DS-AML cases were M7 and 3 were M6. Importantly this was not apparent initially when the provisional diagnoses recorded by the treating hospital were considered. However, following paper review of the MRC AML 10 trial, 6 of the DS-AML cases initially diagnosed at the treating hospital and recorded for trial and study purposes as AML FAB M6 (4 cases), M1 (1 case) and M2 (1 case) were reclassified as AML FAB M7 (4 cases) and M6 (2 cases). The high number of recategorizations prompted review of the non-trial cases of DS-AML. These comprised 5 cases of AML FAB M7 and one case of AML FAB M6. All 6 diagnoses remained the same after review. In contrast, taken together, M6 and M7 accounted for only 18 (8%) of non-DS AML (p<0.001). The mean age at diagnosis of AML was significantly lower in DS children: 2.2 years (95% CI 1.5-3.0) compared with 6.7 years (95% CI 6.1-7.4; p<0.001). Interestingly, the average age at diagnosis of the 18 non-DS children with AML M6 or M7 was only 3.2 years (95% CI 1.1-5.4) comparable with DS-AML. Cytogenetic profiles in these DS-AML cases were consistent with previous reports (3;5,6). Typically, the favorable translocations associated with non-DS AML, namely t(9;21), t(15;17), inv(16), and t(1;22) typically associated with AML FAB M7, were absent. Instead, a variety of unbalanced translocations were seen: t(6;14) (36%) DS-AML; del(6q) in 2/14 (14%); -11 in 1/14 (7%) and +3 in 1/14 (7%) DS-AML cases. Interestingly, both cases of del(6q) occurred in non-trial patients, emphasizing the importance of population based analysis.

We confirm that B-cell precursor ALL (BCP-ALL) is the predominant form of leukemia observed in DS, occurring in 27/54 (79%) cases of DS-ALL compared with 1,067/1,427 (74%) cases of non-DS ALL. T-cell ALL, although reported, is extremely rare. There were no cases of proB or pre-B cell DS-ALL in our study. Age at diagnosis for DS-ALL and non-DS ALL were similar and the differences were not statistically significant. Whilst DS is not a specific entity, ALL in DS appears to be as heterogeneous as ALL in non-DS children. High hyperdiploidy (>50 chromosomes), a good-risk prognostic feature, appears underrepresented occurring in 1/27 (4%) cases. Testing for the most frequent chromosomal abnormality in childhood ALL, the ETV6-RUNX1 fusion resulting from t(12;21), was not standard in the study period. Specific chromosome translocations associated with adverse outcomes in childhood ALL. 11q23/MLL translocations; t(9;22); and t(11;19) were absent in DS cases. However, the numbers are small. The finding of S/27 (19%) cases with loss of 9p is interesting; it has been suggested elsewhere that it might play a significant role in the pathogenesis of DS-ALL. Two of these were in non-trial cases.

Table 1. Characteristics of children with acute leukemia with and without a diagnosis of Down’s syndrome.

<table>
<thead>
<tr>
<th></th>
<th>Acute myeloid leukemia (AML)</th>
<th>Acute lymphoblastic leukemia (ALL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-DS</td>
<td>DS&lt;sup&gt;b&lt;/sup&gt; M6&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Trial entry (%)</td>
<td>180(76.5)</td>
<td>14(77.8)</td>
</tr>
<tr>
<td>Child’s age at diagnosis (yr)</td>
<td>6.7 (6.1-7.4)</td>
<td>3.2 (1.1-5.4)</td>
</tr>
<tr>
<td>Mother’s age at birth (yr)</td>
<td>27.1 (26.4-27.8)</td>
<td>30.0 (25.2-30.0)</td>
</tr>
<tr>
<td>Father’s age at birth (yr)</td>
<td>30.6 (29.7-31.6)</td>
<td>31.2 (28.2-34.1)</td>
</tr>
</tbody>
</table>

Non-DS: non-Dow’s syndrome; DS: Dow’s syndrome. All myeloid leukemia in children with Dow’s Syndrome were FAB M6 (n=3) or M7 (n=1). ‘2 mothers had missing ages because of the child’s adoption.’ 160 fathers had missing ages.

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An excess of AML FAB M7 is well documented in DS: it is a unique disease associated with a pathognomonic mutation of GATA1. This report supports the separate pediatric WHO classification of myeloid leukemia of DS (ML-DS). It is notable that subtypes were reclassified in 6/14 (43%) cases of DS-AML. Unfortunately, this does not appear to be reflected in an earlier report of DS cases in the MRC AML 10 and 12 trials, which appears to rely on the initial classifications, highlighting the importance of ensuring data is as comprehensive and up to date as possible. Here, the final AML subtypes were exclusively FAB M6 and M7. Although an apparent excess of AML M0/7 has been reported, the association is not so clearly described.

A report considering trial cases alone for this period would have missed a third of all cases of acute leukemia in DS, and almost half of all cases of AML in DS. The relatively low entry of children with DS into trials limits the utility of reports solely derived from trials, emphasizing the need for a population-based approach until trial entry rates have improved. Reports suggest that these are increasing, reflecting a growing recognition that children with DS may be successfully treated with intensive chemotherapy and in the context of a trial. There is a pressing need for a collaborative effort to gather data prospectively on all children with DS. The Children with Down’s Syndrome Study (www.dds-study.org) is an observational cohort study set up specifically to address this need and will enable determination of the baseline characteristics of all children with DS. Furthermore, until trial entry improves, a population-based approach is also imperative for the study of children with both DS and leukemia.

Rebecca James, Tanya Lightfoot, Jill Simpson, Anthony V. Atkinson, Eve Romani, Sally Finney  on behalf of the UK Child Cancer Study Investigators, Epidemiology & Genomics Unit, Department of Health Sciences, University of York, York, UK; St James’ University Hospital, Leeds, UK; Northern Institute for Cancer Research, University of Newcastle upon Tyne, UK.

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Keywords: acute leukemia, acute myeloid leukemia, myelodysplasia, myeloproliferative disorder, Down’s syndrome.

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References

Kinetik of bone marrow abrass during induction and achievement of complete remission in acute myeloid leukemia

In acute myeloid leukemia (AML), bone marrow is typically examined 14 days after beginning initial induction therapy. If significant residual blasts remain, the National Comprehensive Cancer Network (NCCN) Guidelines for AML recommend re-treatment. Here we examine whether bone marrow findings on day 21 might modulate the day 14 findings and thus influence the decision to begin a second course.

Our database comprised those 866 adults who had both day 14 and day 21 bone marrows (±2 days) after receiving, from 1995 to 2004, cytarabine (1g/m² per day) con-

Haematological disorders in Down syndrome

Rebecca James
Sally Kinsey

Abstract
Haematological abnormalities are common in children with Down syndrome. These are mostly benign. Neonatal changes include polycythaemia, neutrophilia and thrombocytopenia. In later childhood changes include secondary polycythaemia, red cell macrocytosis, increased red cell distribution width, leukopenia, and immune dysfunctions. Transient myeloproliferative disorder occurs in about 5% and indicates a group at particularly high risk of subsequent leukaemia. A full blood count should be checked in all neonates with Down syndrome. Those with transient myeloproliferative disorder should be discussed with a paediatric haematologist. Of all children with Down syndrome, 1-2% will develop acute leukaemia. Children with Down syndrome and acute myeloid leukaemia usually respond well to chemotherapy. Acute lymphoblastic leukaemia responds less well, and particular care must be taken to minimize risk of infection. There is a paucity of data for both benign and malignant haematological disease, and this needs to be addressed.

Keywords acute myeloid leukaemia; Down syndrome; haematological diseases; myeloproliferative disorders; precursor-cell lymphoblastic leukaemia; lymphoma; transient myeloproliferative disorder

Introduction
The clinical features of Down syndrome (DS) were first described by John Langton Down in 1866. The underlying association with trisomy 21 was recognized in 1959. The number of babies born with DS has recently increased; the incidence of DS in the UK is currently estimated at 1.2 per 1000 live births (National Down Syndrome Cytogenetic Register 2006 Annual Report www.woliusen. gmul.ac.uk/index/reports/NDSCompleteReport06.pdf). DS is associated with a spectrum of medical problems, and children with DS have an improved survival so that this population is of growing importance to both hospital- and community-based paediatricians. Specific scientific advances have drawn increasing attention to haematological disease in DS, but there is still a paucity of information about many conditions.

Benign haematological disorders
Recent interest in DS-related acute leukaemia has somewhat obscured the fact that benign and not malignant haematological disorders are far more common in this population. Indeed, it has long been recognized that haematological parameters differ in children with DS from birth onwards (Table I).

White-cell abnormalities
The first haematological abnormality reported in healthy children with DS was leukopenia. Subsequent studies confirmed this. In the largest published series, 158 full blood counts (FBCs) taken from 266 neonates with DS found a neutrophilia in 80% in the first week. However, there are problems with this study: it is not clear what features precipitated FBCs in those that had them; it was retrospective; there is no follow-up either of repeat counts or of clinical outcomes; there is a lack of information about the type of sample (red-cell parameters in particular may vary depending on whether the sample is venous, capillary or arterial); and finally, there is no morphological information.

Although the clinical implications of mild leukopenia reported in children with DS are unclear, this population does appear to have impaired immune function. Retrospective review of children hospitalized for sepsis indicated an increased mortality in those with DS, and a recent prospective cohort study demonstrated that DS was a risk factor for respiratory-syncytial-virus-related bronchiolitis. An increase in autoimmune diseases – notably coeliac disease, acquired hypothyroidism, thyrotoxicosis, chronic active hepatitis and diabetes mellitus – have also been demonstrated in DS. However, although studies have suggested that there is an altered lymphocyte profile in DS, the basis of any immune defect remains unclear. There is no specific guidance for immunization in children with DS, and until there is they should receive the same regimen as other children.

Red-cell abnormalities
Neonatal polycythaemia: the association between neonatal polycythaemia and DS was first recognized when a study evaluating...
neonates with polycythaemia from a general neonatal population reported a 20-fold excess of DS in those with polycythaemia. Two retrospective reviews then confirmed the association. Neonatal polycythaemia in DS is not associated with concurrent cardiac disease; congenital cardiac disease in DS is not usually cyanotic, and in any case polycythaemia would not usually appear for several days in congenital cyanotic cardiac disease. Umbilical-cord erythropoietin levels, a proxy marker of fetal hypoxaemia, have been shown to be significantly higher in neonates with DS. Interestingly, there is only one report of a prospective longitudinal study of full blood counts in neonates with DS; this study followed only 35 neonates for 12 months. Although 64% of the infants were polycythemic in the first week of life, this resolved after 8 weeks. The pattern of change of haemoglobin was similar to that in infants without DS, falling to a nadir at around 2 months and then rising slowly to reach a plateau. The largest dataset reported found polycythaemia in 35% of DS neonates. Interestingly, polycythaemia was almost three times as likely if thrombocytopenia was present. Limitations to this study have already been discussed.

Red-blood-cell macrocytosis: macrocytosis was first recognized in adults with DS. A retrospective analysis of FBCs taken from a general paediatric population found an excess of children with DS amongst those with macrocytosis. Following this, a prospective case–control study demonstrated a significantly higher mean red-cell volume (MCV) and haematocrit in DS. There was no significant difference in red-cell or serum folate levels, nor was any difference associated with the high MCV. A subsequent study also reported a significantly higher MCV, mean cell haemoglobin concentration (MCHC), red-cell and serum folate, vitamin B12, haptoglobin, red-cell creatine, serum iron, ferritin and hepcidin were not significantly different between the DS group and controls. The high MCV might reflect enhanced erythropoiesis in response to impaired oxidative metabolism. This might occur as a gene dosage effect given that the gene for a superoxide dismutase (SOD1) is located on chromosome 21.

A recent retrospective review compared full blood counts from children with DS to those from two control groups, one with and one without cardiac disease. The MCV and MCH were significantly higher in DS. The haemoglobin and haematocrit were also higher in DS, but this was significant only when compared with the control group without cardiac disease. Interestingly, for children with DS cardiac disease had little effect until 2–7 years of age, when the haemoglobin, MCH and MCHC became significantly higher in those with cardiac disease. The only longitudinal data available on neonates with DS confirmed red-cell macrocytosis and described a rise in MCV over the first year of life. This was not associated with a reticulocytosis, and serial erythropoietin levels were low to normal.

Several papers emphasize the need for DS-specific reference values for haematological parameters. Comparison of full blood counts from a child with DS with standard parameters might be misleading. For example, a child with DS and iron deficiency might have an MCV that is low for DS but which appears normal when compared to standard charts, so that iron deficiency might be missed.

Platelet disorders: thrombocytopenia in DS was first reported in two of three siblings with DS in 1966. Thrombocytopenia has since been consistently demonstrated in DS neonates. In the two studies with follow-up the thrombocytopenia resolved. Neonatal thrombocytopenia appears to be a feature of DS rather than an indicator of ADL or disease. Further, morphological examination of blood films from DS neonates suggests that large platelets are common, so that total platelet volume may be maintained. Marked thrombocytopenia will need discussion with a paediatric haematologist. Thrombocytopenia at a later stage of childhood may herald the development of myelodysplasia (MDS, discussed below) and merits discussion with a paediatric haematologist.

Malignant haematological disorders

The first case report of acute leukaemia in DS was in 1930. Subsequently, a postal survey confirmed a growing suspicion that acute leukaemia occurred with increased frequency in children with DS. Several population-based studies have highlighted the increased risk of acute leukaemia in children with DS. Unfortunately, much of the data is derived from trial series, which may be incomplete and potentially subject to bias; trial entry for children with DS has lagged behind that of the general population. Indeed, data from the UK Childhood Cancer Study—a national population-based study which recorded all diagnoses of childhood cancer and leukaemia in the UK from 1990 to 1994—indicated that only just over two-thirds and only just over half of all children with DS and either acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) respectively, were entered in trials. Until trial entry improves, population-based studies remain a more robust way of examining this high-risk population. All children with DS and suspected acute leukaemia should be referred urgently to a paediatric haematologist.

Transient myeloproliferative disorder

Transient myeloproliferative disorder (TMD), also called transient abnormal myeloipoiesis, or transient leukaemia) is a clonal myeloproliferative disorder of myeloid origin which has been recognized in neonates with DS for over 50 years. The exact incidence of TMD is unknown; historically the diagnosis was not routinely sought, and there are no agreed diagnostic criteria. Typically, there is a high WBC count, with blasts on the blood film, often with thrombocytopenia, a leuko-erythroblastic picture, and megaloblastic haemopoiesis. The overall incidence of 10% is derived from a small series that examined blood films from newborn DS infants. Two recent studies indicate a lower incidence. TMD was diagnosed in 6% of FBCs from DS neonates analysed retrospectively. Retrospective analysis of Guthrie cards from children registered with DS found a GATA1 mutation, and inferred a diagnosis of TMD, in 4% of cases. Typically, TMD is asymptomatic, and the disorder resolves spontaneously within 3 months. However, a minority of patients may experience life-threatening disease with liver fibrosis, pleural and pericardial effusions, and disseminated intravascular coagulation. A child with symptomatic TMD will need urgent referral to a paediatric haematologist. Those with life-threatening symptoms will require treatment, usually with a single course of intravenous cytosine arabinoside. In view of the risk of subsequent AML, a child with asymptomatic TMD should also be discussed.
with a paediatric haematologist, although management may con-
tinue under a general paediatrician. At present, practice varies
around the UK. In view of the high risk of subsequent AML, we
suggest that children with DS and TMD should have FBCs at least
yearly until the age of 5 years. Additionally, their notes should
be clearly marked so that if they re-present in following years
with any FBC abnormality they can be speedily referred to a pa-
ediatric haematologist to exclude leukaemia.

**GATA1 mutations in TMD:** GATA1 is a key DNA-binding tran-
scription factor, encoded on the X chromosome, that regulates
the growth and maturation of erythroid cells, megakaryocytes,
esochnophils and basophils/mast cells. Similarities between acute
megakaryoblastic leukaemia (AMKL), the predominant subtype
of AML in DS, and TMD have long been recognized. The blasts
have a similar immunophenotypic profile. In 2002 an obligate
mutation of GATA1, producing a short form of GATA1 (GATA1s),
was demonstrated exclusively in children with AMKL and DS.
Other groups have since confirmed that a GATA1 mutation is
pathognomonic of AMKL in DS and have also demonstrated simi-
lar mutations of GATA1 in DS neonates with TMD. Examination
of Guthrie cards of children with DS later diagnosed with TMD
and/or AMKL reveals similar GATA1 mutations.

Taken together, it appears that GATA1 mutations, resulting
in GATA1s, are acquired in utero, and are associated with TMD.
Typically, TMD resolves spontaneously, but the GATA1 may
reappear in association with the development of AMKL. A recent
prospective study of 48 newborns diagnosed with both DS and
TMD reported the subsequent development of AMKL in 8/48
(17%) of neonatal TMD. Interestingly, one of the children with
neonatal TMD developed ALL. However, it is not possi-
ble at present to predict which neonates with TMD are likely
to develop subsequent leukaemia, and so all require some degree
of monitoring.

**Acute myeloid leukaemia**

Both myeloid leukaemia of Down syndrome (ML-DS), a specific
disease within this population, and sporadic AML, representing
a heterogeneous group of AML subtypes, appear to be increased in
DS.

**Myeloid leukaemia of Down syndrome:** AMKL occurs around
500-fold more often in children with DS compared with the general
paediatric population. Further, AMKL in DS has several unusual
features: it occurs in the first 5 years of life; it tends to be pre-
ceded by a slowly progressive myelodysplastic prodrone of 4–24
months; there is an obligate GATA1 mutation; and AMKL blasts
are exquisitely sensitive to chemotherapy. Additionally, there is
a suggestion that response to treatment is further improved by
early treatment. Cytogenetic analysis typically shows absence of
favourable chromosomal translocations such as t(8;21), t(15;17),
in 16, t(12;22), although unbalanced translocations – particularly
+8, del eq., +11, +21 – appear to be increased.

Given that the natural history of myelodysplasia in DS is
progression to AMKL, and as there is no biological, prognostic
or therapeutic reason to differentiate them, the term myeloid
leukaemia of DS (ML-DS) was proposed to encompass myelodys-
plasia and AMKL with an obligate GATA1 mutation in children
with DS.

A child with ML-DS should be under the care of a paediatric
haematologist. Given the excellent response to chemotherapy,
this should be offered to all patients. Although children with
DS are not eligible for the current AML 15 trial, interim guide-
lines have been issued pending the international DS AML trial
which is due to open. The guidelines are designed to minimize
morbidity and mortality, which are higher in this population, by
limiting treatment to four courses and by reducing exposure to
anthracyclines.

**Sporadic AML:** myeloid leukaemia in children 4 years of age or
older with Down syndrome often lacks GATA1 mutation, whilst
cytogenetics and risk of relapse are more akin to sporadic AML.
Analysis of AML subtypes by several groups has suggested that
acute erythroleukemia may be specifically increased in
children with DS, whilst other studies have shown a spread of
subtypes. At present, children are treated as described above for
ML-DS.

**Acute lymphoblastic leukaemia**

ALL is at least as common in DS as AML. Although ALL in DS is
a more heterogeneous disease than AML in DS, it is important to
note that the profile of ALL in DS does differ from that in the gen-
eral population. Precursor B-cell ALL is the predominant form,
with T-cell ALL being extremely rare. High hyperdiploidy is typi-
cally underrepresented, and chromosomal translocations associ-
ated with adverse outcomes such as 11q23/MLL, t(9;22), t(1;19)
are often absent. A specific mutation of JAK2 has recently been
demonstrated in DS associated with a distinct ALL phenotype.

Although trial data may miss many cases of DS-ALL, they remain
the main source of data. A review of DS children treated in
MRC ALL 97 demonstrated a significantly worse event-free
survival (EFS) compared with other children (personal communi-
cation, UKALL 2003 coordinators). In particular, there were more
deaths from sepsis than expected. A lower EFS in DS has also
been reported by other groups. In view of this increased mortal-
ity, which has persisted in UKALL 2003, specific recommenda-
tions have been issued. These aim to lower treatment intensity
by omitting or reducing the daunorubicin dose on induction, by
reducing the number of intensive chemotherapy courses, and by
reducing the duration of maintenance for boys so that treat-
ment ends at 2 years for all patients. In addition, guidance for
supportive care has been issued, aiming to diagnose and treat
infections more promptly. Recommendations include consider-
ation of prophylactic antibiotics for DS children receiving intensive
chemotherapy, frequent outpatient review, a low threshold for
diagnosing sepsis, treatment of all episodes of febrile neutropenia
as high-risk, and early involvement of intensive care specialists.

**The future**

Children with DS are susceptible to a spectrum of haematological
abnormalities ranging from asymptomatic changes of the FBC to
acute leukaemia, and this is so from birth onwards. Much of the
literature is based on anecdotal reports or on small and usually
retrospective series. Consequently, answers to many important
questions remain elusive. Until trial entry improves, popula-
tion-based studies remain a more robust way of examining this
high-risk population. The Children with Down’s Syndrome Study
(CDSS, www.ccds.org.uk) is a collaborative project – involving the Down’s Syndrome Association, the Down’s Syndrome Medical Interest Group, clinicians and scientists – set up to address this need. The CDSS is prospectively following a cohort of children with DS from birth onwards, collecting information about all aspects of health from hospital and community medical records, as well as sequential biological samples. It will provide an evidence base for the management of haematological and (other) disorders in DS.

FURTHER READING


Practice points

- Abnormalities of the full blood count are common in children with DS
- Neutrophil counts include polychromasia, neutrophilia and thrombocytopenia
- In later childhood, changes include secondary polychromasia, red-cell macrocytosis, increased red-cell distribution width, leucopoenia, and immune dysfunction
- Transient myeloproliferative disorders (TMD) occur in about 5% of cases and indicates a group at particularly high risk of subsequent leukaemia; A full blood count should be checked in all neonates with DS, and those with TMD should be discussed with a paediatric haematologist
- Between 1 and 2% of children with DS will develop acute leukaemia
- Children with DS and acute myeloid leukaemia usually respond well to chemotherapy
- Acute lymphoblastic leukaemia is harder to treat, and particular care must be taken to minimize risk of infection
Appendix 13. Oral presentation at the 2008 Royal College of Paediatrics and Child Health Spring Meeting.

Abstract reference: RCPCH/SM08/18556

The Children with Down’s Syndrome Study (CDSS): a robust evidence base for the management of children with Down’s syndrome

Aim: To establish a population-based cohort of children with Down Syndrome (DS) and follow them from birth onwards to investigate their health.

Methods: The study is a collaborative venture between clinicians, epidemiologists, laboratory scientists, the Down’s Syndrome Medical Interest Group, and the Down’s Syndrome Association. Following the identification of these key stakeholders, a two-stage wide ranging consultation process was initiated. In the first stage, the emphasis was on understanding clinical practice in relation to the care of children with DS in their early years. In the second stage families of children with DS were visited and their views sought. The target population is covered by six Neonatal Network Regions (~175 000 births per annum) in the North of England. After obtaining ethics approval and conducting a successful pilot in one Neonatal Network area, the study has been extended across the five adjacent Neonatal Networks. All families with a baby with DS born since 1st May 2006 are eligible. The information collected at birth includes a new-born blood sample, as well permission to contact the parents later and to access their medical records. Follow-up includes the completion of annual health questionnaires and the collection of buccal swabs from family members and blood samples from the child.

Results: To date, the study is recruiting in 52 hospitals across the six Networks, and 120 families have been recruited. Comparison with data from the National Down’s Syndrome Cytogenetic Registry for 2006 indicates an initial ascertainment of almost 75%. This will improve as the study becomes established and the remaining 11 hospitals come on-board. We anticipate ~500 families will be enrolled by the end of 2009. Initial analysis will focus on describing haematological parameters and illness patterns.

Conclusion: The CDSS will be the largest population-based cohort of children with DS anywhere in the world. It is a significant resource with the potential to develop an evidenced-based approach to the understanding and management of the health and care of children with DS.

Neonatal Networks - an effective vehicle for improving clinical practice

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Meetings with clinicians
In the process of designing a multi-centre cohort study of children with Down’s syndrome (DS), clinicians in each of the 12 individual hospitals within the Yorkshire Neonatal Network were consulted to find out how neonates with DS were managed.

Variation between clinical practice and perceived best practice identified
It became apparent that it was not routine practice in all hospitals for a full blood count to be checked in a relatively well neonate with known or suspected DS.

Discrepancy discussed at the Yorkshire Neonatal Network Forum
The two main reasons for not checking a neonatal full blood count were:
1. Lack of awareness of the recent advances in understanding the haematology of DS.
2. Lack of confidence in interpreting abnormalities. The reasons why this is now considered best practice were reiterated.

Agreement reached on best solution
Clinicians were keen for the results to be reviewed by a paediatric haematologist so that there would be advice and back-up and it was suggested that it would be simplest to take a sample when cytogenetics were being taken to minimise venepuncture.

Regional Service agreed protocol disseminated via Network mailing
The logistics of the service were agreed with the Pathology and Paediatric Haematology teams. As the service had arisen from clinicians at the Network Meeting it already had grassroots support and awareness.

Why should a full blood count be checked routinely in a neonate with Down’s syndrome?
Haematological abnormalities are common in this group:
• Benign changes including neutrophilia, polycythaemia and thrombocytopenia are present in the majority;
• Up to 10% may have transient myeloproliferative disorder (TMD). This group remain at particularly high risk of developing acute megakaryoblastic leukaemia (AMKL).

Paediatric haematologists confirmed their view that it is now best practice to check a full blood count in all neonates with DS.

What we do now:
A paediatric haematology service is offered to all consultants in the region, who can send a full blood count sample in to the regional centre where the results and a blood film are reviewed. Advice is given as appropriate.
Using Neonatal Networks to facilitate research into high risk groups: the successful establishment of a population based cohort of children with Down’s syndrome

Background:
- The Children with Down’s Syndrome Study (CDSS; www.cdss.org.uk) is a collaborative project set up by the Down’s Syndrome Association, the Down’s Syndrome Medical Interest Group, clinicians and researchers. It follows a cohort of children with Down’s syndrome (DS) from birth onwards, collecting information and sequential biological samples.
- Neonatal Networks (NN) - arose out of a proposal by the Department of Health that hospitals work closely together in formal managed networks to provide a safe and effective service for mothers and babies.
- Target area - As families are initially invited to take part in the CDSS soon after the birth of their baby it seemed appropriate to work through Neonatal Networks. Accordingly, the target area is defined by 6 adjacent Neonatal Networks: Cheshire and Merseyside; Greater Manchester; North Trent; Northern; Trent; and Yorkshire.

Methods:
- Contact with Neonatal Networks - Information about the composition and contact details for each of the 6 NN was obtained from the www.basg.org website. Contact was then made with a representative of each of the NN to discuss the possibility of setting up the CDSS in their area.
- Contact with individual hospitals - Efforts were made to establish contact with clinicians in each hospital within the NNs with the aim of giving a presentation about the CDSS to each Paediatric Department. If the clinicians wished to participate in the CDSS then a local lead would be agreed and Research & Development (R&D) approval sought (MREC approval had already been obtained: 06/MRE09/016).
- Completeness of ascertainment - This was determined by comparison of the study cases with the annual returns from the National Down’s Syndrome Cytogenetic Register (NDSR; www.welsh.smpf.ac.uk/ndscr).

Results:
- Contact with Neonatal Networks - The name of the Lead for all 6 NN was available on the website, but none had contact details. Two of the NN (North Trent and Trent) did provide other contact details (for the NN Coordinator and for the NN website respectively). Following further enquiries to identify the Trust in which each Lead was based contact was made with all 6 NN, resulting in invitations to present the CDSS at 4 NN Meetings.
- Contact with individual hospitals - There are 62 hospitals involved in managing the care of newborns with DS within the target area. To date, presentations about the CDSS have been made to the Paediatric Departments of 61 hospitals. All hospitals have subsequently been invited to participate in the CDSS. The CDSS is fully open in 58 hospitals, and R&D approval is awaited in 2 hospitals. A presentation is yet to be made in 2 hospitals.
- Recruitment
Recruitment opened on 1st May 2006. All children born since then with DS are eligible.
- Completeness of ascertainment
Comparison of cases recruited into the CDSS in 2006 with those notified by cytogenetic laboratories to the NDSR indicate completeness of ascertainment of ~67%.

Conclusion: The existing NN structure, and the information provided about this on the www.basg.org website enabled immediate identification of relevant hospitals, although contact details for individuals within the NN were not easily accessible. The four invitations to speak to NN meetings allowed rapid dissemination of information and consensus about participation across networks.

The positive response of the clinicians in individual hospitals has been tremendous - both in agreeing to participate and in then inviting families to take part. Current recruitment rates are excellent making this a very significant data set with the potential to make an enormous contribution to our understanding and management of the health of children with Down’s syndrome.

The future:
- Geographical expansion - interest in participating in the CDSS has come from across the UK and expansion beyond the initial target area is being discussed.
- Developing collaborations - The Epidemiology and Genetics Unit in the University of York is responsible for running the study and banking the data and samples. Approaches from groups with expertise in different areas of health are now being considered by the CDSS Steering Group in order to maximise the potential of the CDSS.
- Analysis - preliminary analysis of the neonatal blood samples is underway with a view to defining normal ranges for this group.

2. Note that one hospital is listed on the www.basg.org website as being in both the Greater Manchester and the Cheshire and Merseyside Neonatal Networks.


Haematological Reference Ranges for Neonates with Down Syndrome In the UK: A Report From the Children with Down’s Syndrome Study

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Introduction: The Children with Down’s Syndrome Study (CDSS; http://www.cdss.org.uk/) is a unique observational study following a population-based cohort of children with Down’s syndrome (DS) from birth onwards. Here we present the initial set of neonatal full blood count results - demonstrating that haematological values in DS neonates differ markedly from those of the general neonatal population; and providing, for the first time, DS specific reference ranges for haematological parameters for immediate clinical use.

Methods: Recruitment to the population based CDSS opened in May 2006 and is ongoing. At entry, a blood sample taken in EDTA is sent to a designated laboratory with full CPA (UK) accreditation and processed using a Sysmex XE2100 or Advia 2120 according to a standard operating procedure. Clinical and demographic details, including date of birth, gestational age and birth weight are collected routinely. Of the 229 children recruited with appropriate samples, 197 are included in the analysis presented here: 32 were excluded because the sample was after 28 days of age; had a delay in processing; or had no result. Regression analyses considered effects of age at sampling, days until processing, sex, and source of sample. Gestational age and birth weight were examined in children sampled within 3 days of birth.

Results: There were 109 boys and 88 girls. Median age at sampling was 3 days (IQR: 1-7). Median gestational age was 39 weeks (IQR: 37-39) and median birth weight was 3.0 kg (IQR: 2.4-3.4). The DS neonates had a similar RCC, but higher HB, HCT and MCV, when compared with standards for the general neonatal population: RCC 5.4 ± 0.82 x1012/l; HB 20.0 ± 3.3 g/dl with HCT 0.62 ± 0.1%, and MCV 115.5 ± 9.0 fl. By conventional standards, 43% of DS neonates would be considered to be polycythaemic (HCT>65%), and 76% macrocytic (MCV>110 fl). WCC was 13.9 ± 8.4 x109/l with differential analysis (on a subset of
100 children with suitable samples) showing neutrophils \(6.4 \pm 5.3 \times 10^9/\text{l}\); lymphocytes \(3.4 \pm 2.0 \times 10^9/\text{l}\); monocytes \(0.97 \pm 0.55 \times 10^9/\text{l}\); eosinophils \(0.22 \pm 0.32 \times 10^9/\text{l}\); and basophils \(0.20 \pm 0.19 \times 10^9/\text{l}\). In 41% cases monocytes were >1.0 \times 10^9/\text{l}. The overall mean platelet count was \(161 \pm 89 \times 10^9/\text{l}\). Thrombocytopenia was most common in the first week of life: 63% having platelets < 150 \times 10^9/\text{l}. Only 8% of children sampled in their fourth week of life had a platelet count < 150 \times 10^9/\text{l}. Mean MPV was 11.8 \pm 1.0 \text{fl} which is greater than the 95th percentile MPV for a general neonatal population. HB, RCC, HCT, MCV, RDW-CV, nucleated red blood cells, WCC, neutrophils and lymphocytes all fell with each increasing day of age. These parameters did not vary with sex or source of sample. Platelet and monocyte number both increased slightly with increasing birth weight, while HB and RCC fell. HB, HCT, RCC and neutrophils increased with each week of increasing gestational age, while the platelet count fell.

**Conclusions:** The haematological profile of DS neonates appears to be distinct from that of the general neonatal population reflecting altered trilineage haematopoiesis. The most striking features are the increased HB, HCT and MCV - with a preserved RCC; monocytosis; and thrombocytopenia with increased MPV. These features, particularly large haemoglobin-rich erythrocytes and thrombocytopenia, are associated with fetal haematopoiesis, suggesting that the switch to adult haematopoiesis may be delayed in DS. For example, the mean MCV here is comparable with that at 30 weeks gestation for the general population. Interestingly, the neutrophil count appears lower than the general population, although it follows a similar postnatal pattern: falling rapidly over the first few days before plateauing from day 10. Many neonatal units incorporate the neutrophil count in algorithms to indicate the likelihood of sepsis; these may need to be revised for DS neonates. Fetal thrombocytopenia has been described previously in DS. It is clear from this work that the platelet count increases with advancing postnatal age, so that in contrast to other causes of neonatal thrombocytopenia, a well DS neonate with thrombocytopenia does not require additional investigation. This work provides a much needed evidence-base for clinical practice: the predictive percentile charts indicating the expected range by age for the neonatal period and illustrating the natural history.

**Disclosures:** No relevant conflicts of interest to declare.
Appendix 17. Oral presentation at the 2010 American Society of Haematology Annual Meeting; abstract 80.

A Comprehensive Report of Blood Cell Morphology for Neonates with Down’s Syndrome In the UK: A Report From the Children with Down’s Syndrome Study

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¹Epidemiology&Genetics Unit, University of York, York, United Kingdom
²St. James’ Univ. Hospital, Leeds, United Kingdom

Introduction: The Children with Down’s Syndrome Study (CDSS; http://www.cdss.org.uk/) is a unique observational study following a population-based cohort of children with Down’s syndrome (DS) from birth onwards. Although haematological abnormalities are well recognised in DS neonates blood morphology is less well described. This is the first prospective report of blood cell morphology in DS neonates.

Methods: Recruitment to the population based CDSS opened in May 2006 and is ongoing. At entry, a blood sample taken in EDTA is sent to a designated laboratory with full CPA (UK) accreditation. Blood films were reviewed by two Paediatric Haematologists following a specific proforma developed after a series of pilot studies which had identified the range of morphological abnormalities seen in DS neonates. Reporting was subsequently standardised so that inter and intra-operator variability were minimised. Critically, abnormal features were recorded for each individual white cell. All slides reported on here were taken within 28 days of birth and had to be of adequate quality with regard to stain, spread and EDTA change. These were considered independently for cell type.

Results: Microscopic review was performed in slides from 154 DS neonates; 88 of these met the quality criteria for at least one cell type. The most consistent abnormalities were of platelets: giant platelets were present in 78/88 (89%); pale, hypogranular platelets in 61/88 (69%) and megakaryocyte fragments in 30/88 (34%). The most common red blood cell changes were macrocytosis and polychromasia, occurring in ≥5% all red cells in 48/68 (71%) and 33/68 (49%) respectively. Nucleated red blood cells were seen in 34/71 (48%); spherocytes in 28/68 (41%); target cells in 27/68 (40%); Howell Jolly bodies in 26/68 (38%) and basophilic stippling in 9/69 (13%). The neutrophils tended to be hypogranular 35/36 (97%) and/or agranular 6/36 (17%). None were hypergranular. Neutrophil vacuolation was common, occurring in 29/36 (81%) whilst Pelgeroid neutrophils occurred in 12/36 (33%) and

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hypersegmentation in 7/36 (19%). Granulocytic nuclear/cytoplasmic dysynchrony was noted in 25/36 (69%) with the nucleus appearing relatively immature and with the granules often clustering in one area leaving an area of agranular bluish cytoplasm. Lymphocyte morphology was unremarkable. Monocytes were typically vacuolated and often had stellate or elongated nuclei: these features occurred in 30/36 (83%); 27/36 (75%) and 6/36 (17%) slides respectively; no azurophilic granules were seen. Both eosinophils and basophils tended to be dysplastic with abnormal hypogranulation in 18/36 (50%) and 20/36 (56%) respectively. Blasts were seen in 19/36 (53%), but cytoplasmic blebbing was rare 2/36 (6%). In the majority the blast type could not be ascribed.

**Conclusions:** The blood cell morphology of DS neonates described above is distinctive and resembles that typically seen in the fetus. An unusual feature of DS neonates is the spontaneous regression of transient myeloproliferative disorder (TMD). This work suggests that fetal, ie hepatic, haematopoiesis is still significant in DS neonates and supports the idea that TMD arises from a fetal progenitor and regresses as the cellular context changes to turn off hepatic haematopoiesis. Myeloid leukaemia later develops in ~20% who have had TMD. This might occur if a GATA1 mutated fetal progenitor was able to seed and successfully populate the bone marrow. Importantly, although blasts were present in 53%, not all of these had TMD. There is a clear clinical imperative for identifying TMD as it occurs in ~4-6% DS neonates and defines a group at high risk of developing a highly treatable leukaemia within a limited time-frame. This work suggests that full blood count and film review alone are insufficient to identify TMD, and that GATA1 mutation analysis is needed. Such expensive, time consuming and specialized analysis would require a national approach. An important lesson of this work is also the need to standardise morphology reporting: to quantify what is an inherently qualitative and subjective process. The proforma developed here provides a template for others to use in this group and in the general population.

**Disclosures:** No relevant conflicts of interest to declare.
Appendix 18. Oral presentations made to other national and regional meetings.

Interest in the Children with Down Syndrome Study has also led to invitations to present at the following meetings:

08/05/08 Yorkshire Academic Paediatrics Group: Establishing a truly population based cohort - The Children with Down Syndrome Study;

05/06/08 Down Syndrome Medical Interest Group one day symposium and members’ meeting: Research update;

10/11/08 Yorkshire branch of the British Society for Community Child Health Autumn Combined Community and General Paediatric Meeting: The Children with Down Syndrome study;

01/04/09 Guest lecture at the 2009 Royal College of Paediatrics and Child Health Spring Meeting.

20/10/09 Yorkshire Neonatal Network Forum: The Children with Down Syndrome Study;

27/11/09 Down Syndrome Medical Interest Group one day symposium and members’ meeting: Haematology update;

26/11/10 Down Syndrome Medical Interest Group one day symposium and members’ meeting: Haematological reference ranges for babies with Down syndrome;

24/03/11 Yorkshire Neonatal Network: Haematological reference ranges for babies with Down syndrome

In addition I have regularly been invited to present at in-house meetings both at the University of York and within the Leeds Teaching Hospitals.
# List of Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AGA</td>
<td>appropriate for gestational age</td>
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<tr>
<td>ALL</td>
<td>acute lymphoblastic leukaemia</td>
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<tr>
<td>AMKL</td>
<td>acute megakaryoblastic leukaemia</td>
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<tr>
<td>AML</td>
<td>acute myeloid leukaemia</td>
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<tr>
<td>CDSS</td>
<td>Children with Down Syndrome Study</td>
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<tr>
<td>CI</td>
<td>confidence intervals</td>
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<tr>
<td>CLRN</td>
<td>comprehensive local research network</td>
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<tr>
<td>CPA</td>
<td>Clinical Pathology Accreditation</td>
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<td>DGH</td>
<td>district general hospital</td>
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<td>DS</td>
<td>Down syndrome</td>
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<td>DSA</td>
<td>Down Syndrome Association</td>
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<tr>
<td>DSMIG</td>
<td>Down Syndrome Medical Interest Group</td>
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<tr>
<td>EDTA</td>
<td>ethylene diamine tetraacetic acid</td>
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<tr>
<td>ECSG</td>
<td>Epidemiology and Cancer Statistics Group</td>
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<tr>
<td>FBC</td>
<td>full blood count</td>
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<tr>
<td>FEP</td>
<td>free erythrocytic protoporphyrin</td>
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<tr>
<td>GATA1</td>
<td>globin transcription factor 1</td>
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<tr>
<td>GATA1s</td>
<td>short form of globin transcription factor 1</td>
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<tr>
<td>GP</td>
<td>general practitioner</td>
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<td>HB</td>
<td>haemoglobin</td>
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<td>HbA2</td>
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<td>HbF</td>
<td>fetal haemoglobin</td>
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<td>hCG</td>
<td>human chorionic gonadotrophin</td>
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<td>haematocrit</td>
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<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>IHH</td>
<td>Intermountain Healthcare Hospitals</td>
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<td>ISLH</td>
<td>International Society for Laboratory Haematology</td>
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<tr>
<td>I:T</td>
<td>immature to total neutrophil ratio</td>
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<tr>
<td>JAK2</td>
<td>janus kinase 2</td>
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<td>LBW</td>
<td>low birth weight</td>
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<td>MCH</td>
<td>mean cell haemoglobin</td>
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<td>MCHC</td>
<td>mean cell haemoglobin concentration</td>
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<td>MCV</td>
<td>mean red blood cell volume</td>
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<td>MDS</td>
<td>myelodysplasia</td>
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<td>ML-DS</td>
<td>myeloid leukaemia of Down’s syndrome</td>
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<td>MPV</td>
<td>mean platelet volume</td>
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<td>MRC</td>
<td>Medical Research Council</td>
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<td>NDSCR</td>
<td>National Down Syndrome Cytogenetic Registry</td>
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<td>NDSP</td>
<td>National Down Syndrome Project</td>
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<tr>
<td>NEQAS</td>
<td>National External Quality Assessment Scheme</td>
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<td>NHS</td>
<td>National Health Service</td>
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>NRBC</td>
<td>nucleated red blood cells</td>
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<tr>
<td>PCHR</td>
<td>personal child health record</td>
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<td>PCV</td>
<td>packed cell volume</td>
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<td>PET</td>
<td>partial exchange transfusion</td>
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<td>PLT</td>
<td>platelet</td>
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<td>RBC</td>
<td>red blood cell</td>
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<td>RCC</td>
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<td>RDW</td>
<td>red blood cell distribution width</td>
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<td>SD</td>
<td>standard deviation</td>
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<td>SGA</td>
<td>small for gestational age</td>
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<td>SJUH</td>
<td>St James’ University Hospital</td>
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<td>SOD1</td>
<td>superoxide dismutase</td>
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<td>TMD</td>
<td>transient myeloproliferative disorder</td>
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<td>TS</td>
<td>transferrin saturation</td>
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<tr>
<td>UKAS</td>
<td>United Kingdom Accreditation Service</td>
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<td>UKCRN</td>
<td>United Kingdom Clinical Research Network</td>
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<td>VLBW</td>
<td>very low birth weight</td>
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<td>WBC</td>
<td>white blood cell</td>
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<td>World Health Organisation</td>
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<td>York Hospital</td>
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<td>ZCP</td>
<td>zinc complex protoporphyrin</td>
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References


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