EXPLORING THE MORBIDITY AND MORTALITY IN ACROMEGALY

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DECLARATION

This thesis is based on studies performed at the Leeds Centre for Diabetes & Endocrinology of Leeds Teaching Hospitals NHS Foundation Trust, the Leeds Institute of Rheumatic and Musculoskeletal Medicine and the Leeds Institute of Cardiovascular and Metabolic Medicine of the University of Leeds.

The contents of this thesis are original and the work presented is my own, with the exception of the MR image analysis presented in Chapter 4, which was performed by Mike Bowes and has been referenced accordingly.

No part of this thesis has been submitted for the award of any other degree at this or any other institution.

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Abbreviations

AAMs	Active appearance models
ACR	Albumin/creatinine ratio
ACROQoL	Acromegaly quality of life
ACTH	Adrenocorticotropic hormone
ADH	Antidiuretic hormone
ADP	Adenosine diphosphate
AHI	Apnoea-Hypoxia index
AIP	Aryl hydrocarbon receptor interacting protein
APC	Adenomatous polyposis coli
AT-III	Antithrombin-III
AU	Arbitrary units
AUSCAN	Australian/Canadian Osteoarthritis Hand Index
BMD	Bone mineral density
BMI	Body mass index
BP	Blood pressure
cAMP	cyclic adenosine monophosphate
CI	Confidence interval
CRP	C reactive protein
CT	Computed tomography
cXRT	Cranial radiotherapy
d3GHR	Deletion of exon-3 of the growth hormone receptor
DESS-we	Dual-echo at steady-state water-excitation
DIP	Distal interphalangeal
DISH	Diffuse idiopathic skeletal hyperostosis
DM	Diabetes mellitus
DXA	Dual-energy x-ray absorptiometry
ECG	Electrocardiogram
EQA	External quality assessment
ENaC	Epithelial sodium channel
ESC	European Society of Cardiology
FFAs	Free fatty acids
FIPA	Familial idiopathic pituitary adenoma
FSH	Follicle stimulating hormone
g	Gram
GH	Growth hormone

GHRH	Growth hormone releasing hormone
GI	Gastrointestinal
HDL	High density lipoprotein
HOMA	Homeostasis model assessment
HR-QoL	Health-related quality of life
HRT	Hormone replacement therapy
ICC	Intraclass correlation coefficient
IGF-1	Insulin growth factor 1
IGFBP	Insulin growth factor binding protein
IGT	Impaired glucose tolerance
IMRT	Intensity-modulated radiation therapy
IQC	Internal quality control
IQR	Interquartile range
JSN	Joint space narrowing
JSW	Joint space widening
KLG	Kellgren-Lawrence grade
L	Litre
LatTr	Lateral trochlear femur
LBM	Lean body mass
LDL	Low density lipoprotein
LF	Lateral femur
LH	Luteinizing hormone
LP	Lateral patella
LSCM	Laser scanning confocal microscopy
LT	Lateral tibia
LVH	Left ventricular hypertrophy
MA	Maximum absorbance
MAS	McCune Albright Syndrome
mcg	microgram
MCP	Metacarpophalangeal
MedTr	Medial trochlear femur
MEN	Multiple endocrine neoplasia
MF	Medial femur
MID	Minimally important difference
mm	millimeter
MP	Medial patella
MRI	Magnetic resonance imaging

MT	Medial tibia
NET	Neuroendocrine tumour
NHP	Nottingham Health Profile
NO	Nitric oxide
NS	Non-significant
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
OGTT	Oral glucose tolerance test
OR	Odds ratio
OSA	Obstructive sleep apnoea
PAI-1	Plasminogen activator inhibitor-1
PASQ	Patient-assessed Acromegaly Symptom Questionnaire
PD	Proton density
PIP	Proximal interphalangeal
PGWBS	Psychological General Well Being Scale
QoL	Quality of life
RIA	Radio-immunoassay
SD	Standard deviation
SDH	Succinate dehydrogenase
SERMs	Selective estrogen receptor modulators
SF-36	Short form 36
SHBG	Sex-hormone binding globulin
SIR	Standardised incidence ratio
SKF	Skinfold thickness
SMR	Standardised mortality ratio
SRIF	Somatotrophin release inhibiting factor
SRS	Stereotactic radiosurgery
SSA	Somatostatin analogue
SSM	Statistical shape modeling
SSTR	Somatostatin receptor
T4	Tetraiodothyronine
TAFI	Thrombin-activatable fibrinolysis inhibitor
TBS	Trabecular bone score
TBW	Total body weight
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor

TIA	Transient ischaemic attak
TKR	Total knee replacement
t-PA	Tissue plasminogen activator
TSE	Turbo spin echo
TSH	Thyroid stimulating hormone
TSS	Trans-sphenoidal surgery
ULN	Upper limit of normal
VAS	Visual analogue scale
vWF	von Willebrand factor
WHO	World Health Organization
WHR	Waist hip ratio
WOMAC	Western Ontario McMaster Universities Osteoarthritis Index
X-LAG	X-linked acrogigantism
XRT	Radiotherapy

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CHAPTER ONE

INTRODUCTION TO ACROMEGALY

1.1. Historical background

Acromegaly is a rare clinical condition characterized by chronic growth hormone (GH) excess, resulting into body disfigurement and systemic complications. The name of the disease is derived by the Greek words “άκρο” (acro) meaning extremeties and “μέγας” (megas) meaning large. The term acromegaly was first introduced by Pierre Marie, a French Neurologist, in 1886, who described this clinical pathology as: “There is a condition characterized by hypertrophy of the hands, feet and the face, which we propose to be called acromegaly, which means hypertrophy of the extremities (in reality the extremities are swollen during the entire duration of the disease and their volume increase is the most characteristic feature of this disease). Acromegaly is different from myxedema, Paget’s disease or leontiasis ossea of Virchow” [1]. However, records of descriptions which could be in-keeping with acromegaly exist in the medical literature and precede the description given by Pierre Marie. In 1567, Johannes Wier, a Dutch physician, wrote about a female giant and highlighted that she was born of parents of short stature and that when she reached the age of 14 her menses ceased and started to grow significantly [2]. In 1864, Andrea Verga, an Italian Psychiatrist and Neurologist, used the term “prosopo-ectasia” (widening of the face) to describe a female patient due to her large disproportionate face, which he characterized as “almost frightening”. When the patient died from typhus, Dr Verga discovered in the post-mortem examination a sellar tumour at the size of a walnut, displacing the optic nerves [3]. There had been several reports of the presence of pituitary enlargement in patients with acromegaly, however it was not known at the time, whether the enlarged pituitary was the cause or the outcome of the disease [4-7]. The association of acromegaly with a hyperfunctioning pituitary gland was first described by Roberto Massalongo in 1892, who found granulated cells in the pituitary tumour of a patient with acromegaly [8]. Further work published by Carl Benda in 1900 confirmed that acromegaly is due to hyperfunctioning pituitary adenomas containing adenohypophyseal eosinophilic cells [9, 10]. In the following years, knowledge about acromegaly was further enhanced by studies from Harvey Cushing, Percival Bailey and Leo M. Davidoff who reported remission of the clinical symptoms in

patients with acromegaly following hypophysectomy [11, 12]. Acromegaly was the first pituitary pathology to be associated with a hyperfunctioning pituitary adenoma, as well as defined clinically and histologically, with an appropriate treatment strategy.

1.2. Epidemiology

Acromegaly is a rare condition with a reported average prevalence of 86-137 cases per million of population [13-16] and an incidence of 3-4 new cases per million of population per year [17]. However, reports from Belgium and Iceland indicate an incidence of 7.7-10 cases per million per year [18, 19]. Acromegaly affects both men and women with an equal distribution of incidence and has a mean age at diagnosis of 40 years for men and 45 years of age for women [17]. In contrast, the exact disease onset, however, is very difficult to establish, as the acromegalic changes occur in an insidious and slowly progressive fashion, often leading to a significant delay between disease onset and diagnosis.

1.3. Aetiology

In more than 95% of cases, acromegaly results from GH excess due to a benign pituitary adenoma [20]. Extrapituitary acromegaly is extremely rare and is related to ectopic growth hormone-releasing hormone (GHRH) secretion or even more rarely to ectopic GH production [21].

1.3.1. Pituitary-related acromegaly

In the majority of cases (60%) pituitary adenomas causing acromegaly develop from a pure somatotroph cell population, which secretes GH. Depending on the content of secretory granules, these cells are divided in densely granulated (high content of secretory granules, with diffuse immunostaining) and sparsely granulated (low content of secretory granules, with scattered immunostaining). Densely granulated somatotroph adenomas are more commonly seen in older patients (>50 years of age), tend to progress more slowly and respond better to medical therapy. In contrast, sparsely granulated somatotroph adenomas

develop more commonly in younger patients, are more aggressive in terms of tumour growth, with poor response to medical therapy [22, 23].

In approximately 25% of cases pituitary adenomas can co-secrete GH and prolactin. These adenomas may consist of two distinct cell populations, a somatotroph one secreting GH and a lactotroph secreting prolactin [24, 25], or can originate from a single mammosomatotroph stem cell and consist of a monomorphic population of cells, which secrete both GH and prolactin [26]. Rarely somatotroph adenomas can be plurihormonal and express GH as well as prolactin, corticotropin (ACTH), thyroid-stimulating hormone (TSH) and α-subunit at different combinations, which can lead to a mixed clinical presentation with symptoms of acromegaly, as well as symptoms of hyperprolactinaemia, Cushing's disease and thyrotoxicosis respectively [27-29].

Silent somatotroph adenomas are positive for GH on immunostaining, however they are clinically non-functioning and do not cause the systemic features which are associated with acromegaly. These tumours are diagnosed following surgical resection for localized mass-effect symptoms, rather than symptoms related to GH excess. Serum GH and insulin-like growth factor 1 (IGF-1) are usually normal, however in some cases a modest increase in GH and prolactin levels can be observed [30, 31].

Malignant GH-secreting pituitary tumours are exceptionally rare. These tumours are aggressive and associated with poor prognosis. GH levels are often grossly elevated and the tumours fail to respond to conventional therapy. Pituitary carcinomas tend to be locally invasive tumours, however the presence of metastatic disease is required in order to support the diagnosis of malignant pituitary tumour [32-34].

1.3.2. Extrapituitary acromegaly

Ectopic acromegaly is rare, accounting for less than 1% of all cases of acromegaly and is almost always the result of growth hormone releasing hormone (GHRH) secretion more commonly due to neuroendocrine tumours and particularly lung carcinoid tumours or pancreatic cell tumours [21, 35]. Other tumours which have been associated with GHRH

expression resulting in acromegaly include hypothalamic tumours (gangliocytoma, hamartoma, glioma) [36], phaeochromocytoma, adrenal adenoma, small cell lung cancer, medullary thyroid cancer, endometrial and breast cancer [37, 38]. Ectopic GH secretion by a peripheral tumour has only been described in an extremely limited number of case reports and has been associated with a neuroendocrine tumour (NET) or non-Hodgkin's lymphoma [39, 40]. There are also cases of ectopic pituitary tissue in the sphenoid sinus, sphenoid wing, petrous temporal bone and nasopharyngeal cavity, which have been associated with GH hypersecretion [41, 42].

Differentiating between pituitary and ectopic acromegaly can impose significant diagnostic challenges. Clinically, patients present with classic symptoms and signs of acromegaly and therefore symptomatology is not helpful to distinguish between the two pathologies, unless the patient develops clinical features which would be unexpected in an acromegalic patient and indicative of an ectopic source (i.e. respiratory wheeze, dyspnoea, flushing, symptoms related to metastatic disease, which could suggest the presence of a neuroendocrine tumour).

Equally, biochemistry is similar in both pituitary and ectopic acromegaly, characterised by elevated IGF-1 and GH levels, with the latter failing to suppress following an OGTT. However, serum GHRH has been proposed as a useful diagnostic tool (increased GHRH level are seen in ectopic acromegaly versus low levels in acromegaly of pituitary origin) [43]. An additional diagnostic tool for ectopic GHRH secretion is the detection of GHRH positive cells in the tumour. However, this immunocytochemistry technique is not available routinely.

Pituitary imaging does not always allow differentiation between ectopic and pituitary-related acromegaly. Normal volume pituitary or global pituitary hyperplasia are the expected findings from pituitary imaging in ectopic acromegaly, however differentiating between pituitary hyperplasia and adenoma based on imaging is not always possible [43].

Diagnosing ectopic acromegaly is pivotal in the management of the patient, as excision of the responsible tumour is usually curative, whilst avoiding unnecessary interventions to the pituitary preserves normal pituitary function. When surgery is contraindicated or in case of

metastatic disease, somatostatin analogues can be an alternative option to achieve biochemical disease remission with normalisation of GH and IGF-1 levels [43].

1.3.3. Genetic forms of acromegaly

Acromegaly has been associated with a variety of genetic syndromes.

1.3.3.i. McCune Albright Syndrome (MAS)

This is a rare syndrome, with an estimated prevalence between 1 in 100,000 and 1 in 1,000,000 people [44], characterized by the triad of polyostotic fibrous dysplasia, café-au-lait spots and precocious puberty [45]. A variety of endocrinopathies have also been associated with this syndrome, including hyperthyroidism [46], GH excess [47, 48], Cushing's syndrome [49] and renal phosphate wasting [50]. MAS is due to activating mutation of the GNAS gene, activating the G-signaling complex and increasing intracellular cyclic adenosine monophosphate (cAMP) [51]. The GNAS gene can be found in many tissues including bone, skin and endocrine system. In the endocrine system in particular, cAMP can increase hormone synthesis and secretion.

GH excess has been found in approximately 20% of patients with MAS [48]. Identifying and treating these patients is important, as raised GH levels can aggravate craniofacial fibrous dysplasia [48]. Pituitary surgery is extremely difficult in these cases due to involvement of the skull base with fibrous dysplasia. Other treatment options include long-acting somatostatin analogues [48], the use of the GH-receptor antagonist pegvisomant or radiotherapy to the pituitary gland [52, 53]. Occasionally a rise in prolactin levels can also be observed in patients with GH excess [44] and therefore dopamine agonists may also have a role in the treatment of patients with MAS.

1.3.3.ii. Multiple Endocrine Neoplasia

GH-secreting pituitary adenomas can be part of Multiple Endocrine Neoplasia type 1 (MEN-1) syndrome, which also encompasses tumours of the parathyroid glands and pancreas [54].

Clinical suspicion is usually raised when tumours arise in 2 or more endocrine glands in the same patient and the diagnosis is confirmed by identifying a germline MEN-1 mutation via genetic testing. This is an autosomal dominant syndrome with high penetrance, related to mutations inactivating the MENIN tumour suppressor gene, which is located on chromosome 11q13 and encodes menin protein, which is involved in cell proliferation and gene transcription of cell cycle regulators [55, 56]. MEN-1 syndrome has a low prevalence of 1 case in 30,000 individuals [57]. Primary hyperparathyroidism is the most common clinical manifestation of MEN-1 syndrome, present in more than 95% of the affected patients [58]. Pituitary tumours are present in 15-50% of patients with MEN-1 according to different studies [59]. Approximately 60% of these pituitary tumours produce prolactin, while approximately 25% secrete GH, with a higher proportion of plurihormonal adenomas compared with non-MEN1 pituitary tumors [60]. Additionally, MEN-1-related pituitary tumours tend to be larger in size and more aggressive in clinical behaviour compared with sporadic non-MEN-1 pituitary tumours [60]. An association has also been described between MEN-1 and ectopic GHRH secretion causing acromegaly. In a French series, MEN-1 syndrome was diagnosed in 19 out of 25 cases of GHRH-secreting pancreatic tumours [43].

Mutations in the MEN-1 gene are found in up to 90% of patients with a clinical phenotype suggestive of MEN-1 syndrome [56, 59]. In some of the remaining cases, mutations in the cyclin-dependent kinase inhibitor 1B (CDKN1B) gene have been identified. CDKN1B is a tumour suppressor gene, which encodes p27, inhibiting cell cycle progression [61, 62]. This syndrome has been named as multiple endocrine neoplasia type 4 (MEN-4) and screening for mutations in the CDKN1B gene is recommended in patients with MEN-1 phenotype but negative for mutations in the MEN-1 gene [59]. From the limited number of cases reported in the literature (<50) to this date, primary hyperparathyroidism is the most common endocrinopathy [63]. Pituitary involvement has also been described, with 5 cases of acromegaly reported in the context of MEN-4 syndrome [64].

1.3.3.iii. Carney Complex

Carney complex is a rare syndrome comprising of cutaneous and mucosal pigmentation, myxomas of the heart, skin and breast and endocrine tumours, including primary pigmented nodular adrenocortical disease (causing ACTH-independent hypercortisolaemia), pituitary adenomas, thyroid, testicular and ovarian lesions [65]. It is inherited in an autosomal dominant fashion, with high penetrance and is due to germline inactivating mutations of the PRKAR1A gene, located in the long arm of chromosome 17, which encodes the type 1 α-subunit of protein kinase A. Mutations of the PRKAR1A gene increase the cAMP-protein kinase A-mediated intracellular signaling, which is involved in a number of cellular functions that regulate transcription factors and cell proliferation [66].

Regarding the pituitary manifestations of Carney complex, approximately 75% of these patients have increased levels of GH and IGF-1; however only about 12% develop clinical acromegaly [65]. Pituitary adenomas can be single or multifocal and are often surrounded by areas of mammosomatotroph hyperplasia [67]. In those cases, a complete hypophysectomy rather than a selective adenomectomy is required in order to achieve disease remission [68, 69]. Co-secretion of GH and prolactin is common in patients with Carney complex. In patients with biochemical evidence of GH excess, but absence of a discrete pituitary adenoma, medical therapy is indicated [68].

1.3.3.iv. Pituitary adenoma with paraganglioma/phaeochromocytoma syndrome

Mutations affecting the succinate dehydrogenase (SDH) complex are the most common genetic cause of paragangliomas/phaeochromocytomas. These mutations have also been identified in patients diagnosed with a pituitary adenoma as well as paraganglioma/phaeochromocytoma (3PAs syndrome) [70]. In the context of the familial 3PAs syndrome, GH-secreting pituitary tumours have been associated with SDHA, SDHB and SDHD mutations [64]. Sporadic cases of 3PAs syndrome, which do not harbour mutations in the SDH complex genes have also been reported [64, 70].

1.3.3.v. Familial Acromegaly

Cases of acromegaly have been identified in families with familial isolated pituitary adenoma (FIPA) syndrome, which is defined as the presence of pituitary tumours in at least two members of the same family [71]. It is a heterogeneous condition, as different types of pituitary adenomas can occur in different members of the same family [72]. GH-secreting adenomas is the most common pituitary tumour in FIPA families [71]. The genetic cause for the development of pituitary adenomas remains unknown in most FIPA families, however in approximately 20% of those families, mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene have been identified [71, 73]. The AIP gene is located at chromosome 11q13 and is considered to be a tumour suppressor gene, with the majority of the mutations causing a missing or truncated protein, which leads to activation of the cAMP pathway and alteration of the somatostatin receptor downstream pathway, although the exact mechanism of tumorigenesis in the context of AIP gene mutations remains unclear [74, 75].

GH-secreting adenomas is the most common type of pituitary tumour in patients who carry an AIP gene mutation, representing almost 80% of these cases [71]. When compared to non-AIP pituitary adenomas, these tumours are more frequently macroadenomas, with extrasellar invasion, higher levels of GH secretion and worse treatment response rates [73]. There is also a male predominance in AIP-related pituitary tumours and they tend to get diagnosed at a younger age, with approximately 32% of patients with AIP-positive GH secreting adenomas presenting with gigantism, rather than acromegaly [73].

The exact penetrance of the AIP gene mutations is not known, but it is considered to be low [76]. Sporadic cases of AIP gene mutations have also been reported, particularly in young patients (<30 years of age) with macroadenomas [71]. The absence of positive family history of acromegaly in these cases is likely due to the incomplete penetrance of the AIP gene mutations, rather than due to a de novo mutation. Therefore screening of the first-degree relatives of patients with acromegaly related to a sporadic AIP mutation is indicated [77].

Another form of familial acromegaly is related to microduplications on chromosome Xq26.3, which is also known as X-linked acrogigantism (X-LAG). Of the four genes identified in the

duplicated area of the DNA, the GPR101 gene, which encodes for an orphan G-protein coupled receptor, was found to be overexpressed in these patients [78]. X-LAG is a more rare cause of familial acromegaly than AIP-gene mutations, but unlike AIP mutations, X-LAG cases are mostly de novo [79].

1.3.3.vi. Gigantism

Gigantism refers to GH excess which occurs before the closure of the epiphyseal growth plates, leading to tall stature, which is >3 standard deviations above the mean height for the individual's age. Similar to acromegaly, gigantism is caused by a GH-secreting pituitary adenoma, which is related to a genetic syndrome (AIP, X-LAG, MEN-1, McCune-Albright, Carney Complex, MEN-4) [80]. AIP-mutations and X-LAG are responsible for the majority of cases of gigantism. X-LAG in particular, causes a distinctive and clinically severe phenotype, with onset of excessive somatic growth at a mean age of 1-year-old, which is significantly earlier compared with other cases of gigantism [81]. Apart from tall stature, children with this mutation also had enlargement of their extremities and coarsening of their facial characteristics. GH and IGF-1 are markedly elevated in X-LAG syndrome and more difficult to control with medical treatment [81]. Pituitary tumours are usually large and invasive and histology following pituitary surgery shows presence of an adenoma, however pituitary hyperplasia has also been reported [81].

1.4. Growth Hormone Physiology

1.4.1. Growth Hormone Structure and Function

Growth hormone is a peptide hormone produced by the somatotroph cells, which are localized mainly in the lateral wings of the anterior pituitary and comprise approximately 35-45% of all pituitary cells. The human GH genome is located on chromosome 17q22-24 and consists of 5 genes, which encode two forms of GH; a 22-kDa peptide, which contains 191

amino-acids and 4 α-helices, consisting almost 90% of the circulating GH; and a 20-kDa peptide, containing 185 amino-acids and accounting for 10% of the circulating GH [82, 83].

GH is secreted in an episodic, pulsatile fashion, with each pulse lasting for approximately 30 minutes. Secretory pulses are separated by periods of minimal basal GH release, during which serum GH levels are undetectable by conventional immunoassays. GH secretion also follows a circadian pattern, with almost 70% of the total daily GH release occurring nocturnally during the first episode of deep-wave sleep [84]. In contrast, in patients with acromegaly there is blunting of the pulsatile GH secretion, with reduction of the night to daytime GH release ratio and an increase to the cumulative daily GH secretion. A key feature of the GH secretory pattern in acromegaly is that serum GH concentrations are never below the detection threshold of the conventional immunoassays [85].

The actions of GH to the peripheral tissues are mediated directly via the respective GH receptors and indirectly via induction of insulin-like growth factor 1 (IGF-1) primarily from the liver, which acts in both an endocrine and autocrine/paracrine fashion to regulate tissue growth [86]. IGF-1 is also synthesized in extrahepatic tissues including bone, muscle and kidney. It is a 70 amino-acid peptide and more than 99% of the circulating IGF-1 is bound to the IGF-1 binding proteins (IGFBP) 1-6, resulting in the long half-life of IGF-1 [87]. GH and IGF-1 act synergistically to promote linear skeletal growth during childhood and adolescence. GH also regulates glucose homeostasis and lipid metabolism, affecting body composition by increasing and preserving lean body mass and also reducing total body fat mass [88, 89]. Additionally, GH is also implicated in the bone metabolism and contributes to the individual's peak bone mass [90].

1.4.2. Regulation of Growth Hormone Release

GH secretion is regulated through a complex interaction among hypothalamic hormones [growth hormone releasing hormone (GHRH) and somatotrophin release inhibiting factor (SRIF) or somatostatin], circulating levels of IGF-1 and growth hormone segretagogues (mainly ghrelin). Somatotroph cells express specific receptors for GHRH, somatostatin

(somatostatin receptors 1-5 or SSTR1-5) and GH secretagogues [91-93]. GHRH and somatostatin have opposing effects on the somatotroph cell. GHRH promotes GH gene transcription, GH synthesis and secretion [94], while somatostatin inhibits both basal and pulsatile GH release, acting primarily via the SSTR2 and SSTR5 receptors [92, 93]. Although somatostatin suppresses both the amplitude and frequency of the GH pulses, it has no effect on the GH synthesis [95]. Pulsatile GH release is the result of the coordinated and synergistic effect of GHRH and somatostatin, which are both secreted in independent waves from the hypothalamus. GHRH is primarily responsible for the GH pulses, while somatostatin produces the GH troughs. However, the interaction between GHRH and somatostatin is even more complex, as somatostatin increases the sensitivity of the somatotroph cells to GHRH and in a way prepares the somatotroph cell during a GH trough to respond to the subsequent GHRH pulse, optimizing GH release [96].

Ghrelin is a 28-amino acid peptide, produced primarily in the periphery by neuroendocrine cells of the gastric mucosa of the fundus, but also centrally in the hypothalamus and pituitary [97]. Ghrelin is known as the “hunger hormone”, as it has an important role on metabolism and stimulating appetite. It acts via the GH secretagogue receptor and regulates GH release at both hypothalamic and pituitary level, inducing GH secretion [97, 98]. Additionally, ghrelin has a synergistic effect with GHRH on enhancing GH secretion, by increasing GH responsiveness to GHRH [99].

Circulating IGF-1 provides negative feedback to the hypothalamus and pituitary [100] and reduces GH gene expression and GH release [101]. The down-regulation of GH by IGF-1 is achieved via a number of different mechanisms. IGF-1 inhibits the GHRH-mediated GH release at the pituitary level [100, 101]. Additionally, studies in GH-deficient rodents have shown that administration of IGF-1 reduces GHRH release, expression of GHRH receptors and also increased somatostatin expression [103, 104].

1.5. Clinical Features of Acromegaly

Outside the context of a genetic syndrome associated with acromegaly (i.e. MEN-1, MEN-4, McCune Albright Sydrome, Carney Complex), clinical signs and symptoms of acromegaly are associated either to the local mass effects of the pituitary tumour or the systemic actions of GH and IGF-1 excess on peripherals tissues and organs.

1.5.1. Localised tumour-related symptoms

Depending on the size and the invasion of the pituitary tumour, these symptoms often include headache and visual field deficits, when there is significant suprasellar tumour extension with compromise of the optic chiasm [105]. Headaches are not related to the tumour size and have also been reported by patients with small size pituitary adenomas. They can often be severe, requiring treatment with short-acting somatostatin analogue subcutaneous injections for symptomatic relief. Tumour invasion into the cavernous sinuses can impinge upon the III, IV and VI cranial nerves causing diplopia, as well as cranial nerve V causing facial pain. Inferior invasion of the tumour into the sphenoid bone can lead to cerebrospinal fluid leak into the nose and rhinorrhoea.

1.5.2. Symptoms related to the systemic actions of GH/IGF-1

1.5.2.i. Skeletal manifestations / physical appearance

The most typical clinical manifestations of acromegaly are related to skeletal changes and soft tissue hypertrophy under the effect of GH/IGF-1 excess and include enlargement of the hands and feet (patient may complain about increase in the ring or shoe size), coarsening of the facial features with mandibular overgrowth and prognathism, jaw malocclusion, enlargement of nose and tongue, teeth separation and frontal bossing. Changes in physical appearance may occur insidiously over several years and often remain unnoticed by patients, causing significant delay in the diagnosis of acromegaly. It has been estimated that the time between onset of symptoms of acromegaly and diagnosis varies between 6.6 and 10.2 years [106].

Review of patients' old photographs may help to identify slowly progressing changes in physical appearance.

1.5.2.ii. Arthropathy

Arthropathy is the most common complication of acromegaly and cause of morbidity, affecting up to 77% of patients with acromegaly based on symptomatology and clinical criteria (arthralgia, joint stiffness) [107, 108]. However, up to 99% of patients have radiological evidence of arthropathy [109]. Acromegalic arthropathy affects both the appendicular and axial skeleton, including weight and non-weight bearing joints [109, 110]. Common radiological findings from joint imaging include joint space widening and osteophytosis, with joint space narrowing being present in a lesser number of patients and particularly in those with advanced, end-stage joint disease [111]. Arthropathy is one of the main predictors of quality of life (QoL) in patients with acromegaly, with arthralgia being associated with worse health-related QoL outcomes [107, 112]. Arthropathy persists even after successful treatment of acromegaly and biochemical disease control, remaining one of the unresolved issues in patients with acromegaly in the long-term. This is discussed further in Chapter 3.

1.5.2.iii. Cutaneous manifestations

A number of skin manifestations have been described in patients with acromegaly. With GH receptors present in all cell groups comprising of the skin and IGF-1 receptors existing on epidermal keratinocytes [113], GH/IGF-1 excess can affect the dermis, sebaceous and sweat glands as well as hair growth. Hyperhidrosis and oily skin are common findings, present in up to 70% of patients at the time of diagnosis [105]. Other cutaneous manifestations include skin thickening and puffiness associated with deposition of glucosaminoglycans [114], particularly at the face, hands and feet, causing increased facial wrinkles, deepening of nasolabial folds, thickening of heel pads and hardening of the nails [115]. Increased hair growth, independent of androgens has also been reported [115], as well as hirsutism in some female patients with

acromegaly, which is thought to be related to a reduction in the sex-hormone binding globulin (SHBG) due to GH excess, while serum androgen levels remain normal [116]. Skin tags are another common cutaneous manifestation of acromegaly and its presence has been associated with colonic polyps [117].

1.5.2.iv. Neuromuscular symptoms

Nerve entrapment syndromes can develop in acromegaly and are related to enlargement of peripheral nerves, thickening of local periarticular tissues, localized oedema and fluid retention. Carpal tunnel syndrome, due to medial nerve entrapment is the commonest manifestation of the peripheral nervous system, present in up to 50% of patients with acromegaly [118, 119]. Cubital tunnel syndrome, due to neuropathy involving the ulnar nerve at the cubital tunnel has also been reported in patients with acromegaly [120]. Reduction in the degree of nerve enlargement has been demonstrated with successful treatment of acromegaly [121]. Proximal myopathy characterized by muscle pain, proximal muscle weakness and abnormal findings occasionally on needle biopsy and electromyography has also been reported [122].

1.5.2.v. Cardiovascular complications

Cardiovascular disease remains one of the leading causes of mortality in patients with acromegaly. The spectrum of cardiovascular complications in relation to GH/IGF-1 excess includes left ventricular hypertrophy (LVH), cardiomyopathy, cardiac arrhythmias, cardiac valve disease and arterial hypertension [123].

1.5.2.v.1. GH/IGF-1 effects on cardiac physiology

GH and IGF-1 receptors are expressed in abundance in the myocardium and the blood vessels [124]. Studies in animal models and human myocardial tissue have provided an insight into the effects of GH and IGF-1 excess on the myocardium. These include cardiac hypertrophy, with increase to the left ventricular weight and increase to the myocardial contractility,

producing an overall positive inotrope effect on the cardiac function of mice exposed to supraphysiological doses of GH and IGF-1 [125]. Further animal studies showed that this positive inotropic effect is mediated via IGF-1 by increasing the sensitivity of the myofilament to intracellular ionized calcium (Ca^{2+}). In contrast GH did not have a similar effect on myocardial contractility [126]. Studies in human myocardium have also demonstrated that IGF-1 has a positive inotropic effect by increasing the intracellular Ca^{2+} levels [127]. Similarly, administration of recombinant human IGF-1 in patients with heart failure as well as healthy volunteers resulted in increase in stroke volume and cardiac output [128, 129].

In addition to the trophic effects of GH/IGF-1 on the myocardium, there is also evidence that GH and IGF-1 may act as inhibitors of cardiomyocyte apoptosis. In transgenic mice, in which myocardial ischaemia was induced, overexpression of IGF-1 prevented myocyte death in the viable myocardium adjacent to the area of infarction [130]. Similarly administration of IGF-1, one hour prior to ischaemia in a murine model, reduced myocardial injury and attenuated myocyte apoptosis following myocardial ischaemia and reperfusion [131].

Another effect of GH/IGF-1 on the cardiovascular system is via the regulation of peripheral vascular resistance. Administration of IGF-1 in patients with heart failure had a positive haemodynamic outcome, reducing systemic vascular resistance and pulmonary artery wedge pressure [128]. Direct infusion of GH into the brachial artery of healthy volunteers produced an increase in the forearm blood flow, accompanied by a reduction in the forearm vascular resistance [132]. IGF-1 acts as a vasodilator by promoting the release nitric oxide (NO) from endothelial cells [133] and vascular smooth muscles cells [134] and also by reducing vascular tone through increasing the activity of the Na^+/K^+-ATPase in vascular smooth muscle cells [135], whereas GH has been shown to affect the gene expression of the K_{ATP} channel in vascular smooth muscle cells reducing vascular resistance and blood pressure in hypophysectomised rats treated with recombinant human GH for two weeks [136]. GH-mediated release of NO has also been demonstrated in human studies [132].

1.5.2.v.2. Cardiomyopathy

The results of a previous autopsy study performed in 27 patients with acromegaly showed that left ventricular hypertrophy (LVH) and interstitial myocardial fibrosis were the most common characteristics of the acromegalic cardiomyopathy, present in 92% and 85% of the patients respectively [137]. However, this study was conducted long before the recent therapeutic advancements in the management of acromegaly. In the more recent studies evaluating cardiac function using echocardiography the prevalence of LVH ranges between 11% and 78% [138-144]. The significant variability in the frequency of LVH is possibly related to the heterogeneity in the methodology of the different studies regarding echocardiographic criteria used to define LVH and difference in the study populations. More recent studies using cardiac MRI, which is considered the gold standard method of assessing cardiac structure and function [145], found significant discrepancies in the estimated prevalence of LVH between echocardiography and cardiac MRI. A study by Bogazzi et al. showed that echocardiography significantly underestimated the prevalence of LVH compared with cardiac MRI (36% vs. 72% respectively) in a cohort of 14 patients with untreated, active acromegaly [146]. In contrast, a study from a research group in Brazil, found low prevalence of LVH of only 5%, based on cardiac MRI, in 40 patients with active acromegaly prior to the initiation of medical therapy with long-acting octreotide. Echocardiography overestimated the prevalence of LVH in this study, suggesting that it was present in 31% of the patients [147]. This significant discrepancy in the LVH prevalence between the two cardiac MRI studies is related to the different diagnostic criteria applied to define LVH, with the Brazilian group using a higher reference value for the normal cardiac mass. However, both studies reported low rates of cardiac fibrosis, which are considerably lower to the prevalence of cardiac fibrosis shown in previous autopsy study [137].

Diastolic dysfunction has been frequently observed in a number of echocardiography studies in patients with acromegaly, with a prevalence that ranges between 11% and 58% [141, 143, 148, 149]. Diastolic dysfunction in the context acromegaly is considered to be mild and reversible with medical therapy and reduction in the cardiac mass [141, 148]. Systolic

dysfunction has been a rare finding in clinical studies using either echocardiography or cardiac MRI with a frequency of up to 8.3% [138, 139, 143, 146-148]. In the two cardiac MRI studies, despite the difference in the rates of LVH, no cases of systolic dysfunction were reported and all patients had left ventricular ejection fractions within normal limits [146, 147]. Based on data on 943 patients with acromegaly from the French Acromegaly Registry, the rate of heart failure was overall low at 1.9% [150]. The low prevalence of left ventricular systolic dysfunction and heart failure are consistent with data from animal studies showing that GH and IGF-1 have a positive inotrope effect, enhancing myocardial contractility [125-127].

1.5.2.v.3. Heart valve disease

A number of studies have shown increased prevalence of heart valve disease in patients with acromegaly [151-153], with aortic regurgitation being the most common form, present in 30% of patients, followed by mitral regurgitation present in 5% of cases [151]. Regurgitant valvular disease is more prevalent in patients with a history of longer duration of active acromegaly, irrespective of the disease status at the time of the echocardiographic assessment [151-153]. Additionally, increased prevalence of aortic root ectasia has also been reported in patients with acromegaly (up to 26%), which may be contributing to high rates of aortic regurgitation compared with controls [154, 155]. It has been speculated that the heart valve disease in acromegaly is due to alterations in the composition of extracellular matrix due to an increase in the transcription of matrix metalloproteinases genes, caused by a GH-mediated increase in pro-inflammatory cytokines. The changes in the matrix composition can lead to thickening and remodeling of the cardiac valves, resulting in valve incompetence [151]. Although LVH and diastolic dysfunction can improve following when remission of acromegaly is achieved with either surgery or medical treatment [156-159], established heart valve disease does not reverse with successful treatment of acromegaly [151].

1.5.2.v.4. Ischaemic heart disease

Acromegaly is associated with a number of complications including hypertension, increased insulin resistance/diabetes and dyslipidaemia, which are well-recognized risk factors for coronary artery disease [123, 160]. Data from different European Acromegaly Registries, report a prevalence of myocardial infarction, which ranges from as low as 0.7% [150] to 7.5% [161]. Additionally, data from multicenter study in Germany and Denmark did not demonstrate increased risk of myocardial infarction in patients with acromegaly compared to the general population, with a standardized incidence ratio of 0.89 [162] and hazard ratio of 1.0 [163], respectively.

The 10-year risk of developing coronary artery disease, as this predicted by the Framingham score or the European Society of Cardiology (ESC) risk score and the risk of arteriosclerosis, as this quantified by the Agatston score which is a measure of coronary artery calcium, have been evaluated in patients with acromegaly in prospective studies [164, 165]. Using the Framingham scoring system, 27% and 2% of patients were identified as having intermediate and high risk respectively of developing coronary heart disease [164], whereas with the use of the ESC score 16% of patients were considered high risk, with the remaining 84% being in the low-risk group [165]. No difference in the coronary artery calcium content has been found between patients and controls [166], and in fact one study has shown significantly lower Agatston score amongst patients with acromegaly compared with controls [165]. Disease activity status does not influence either the Framingham or Agatston scores [164, 166], implying the presence of coronary artery disease in patients with acromegaly is possibly related to the disease secondary complications of hypertension, diabetes and dyslipidaemia, rather than disease activity. Finally, no ischaemic cardiac events were observed during a follow-up period of up to 5 years [164, 165], suggesting that the overall risk of coronary artery disease in acromegaly is low [165, 166].

1.5.2.v.5. Cardiac arrhythmias

A number of cardiac rhythm abnormalities have been described in patients with acromegaly. LVH and myocardial fibrosis, as previously described in autopsy studies [137], may contribute to an increased arrhythmogenic potential in patients with acromegaly, although presence of myocardial fibrosis was not confirmed by more recent MRI studies [145, 146]. 24-hour Holter monitoring has shown high rates of isolated and paired ventricular and supraventricular ectopies in up to 89% of patients with acromegaly, however no episodes of sustained arrhythmias or arrhythmia-related symptoms were observed [167]. In contrast increased prevalence and severity of ventricular arrhythmias has been reported in a case-control study, however, the rate of LVH in this particular cohort was higher than previously reported [168]. Increased QT dispersion, a measure of the heterogeneity in the ventricular repolarization and a proposed marker of increased potential for ventricular arrhythmias, has been observed in patients with active acromegaly, but not in patients with disease remission, compared with controls [169]. Additionally, increased rate of late potentials, which refers to the presence of low amplitude and high frequency signals at the end of the QRS complex and has been used as a marker of increased risk for ventricular tachycardias, was found in 51 patients with acromegaly and was associated with premature ventricular complexes, although no episodes of sustained ventricular arrhythmias were recorded on 24-hour Holter monitoring of these patients [170].

1.5.2.v.6. Arterial hypertension

In a number of acromegaly registry studies, hypertension in the most common cardiovascular complication observed in 28.8-40% of patients [144, 150, 161, 171]. However, it is possible that these large observational studies have overestimated the prevalence of hypertension in acromegaly, as the results were often based on single clinical measurements of blood pressure (BP). Studies in which 24-hour ambulatory BP monitoring was applied showed a prevalence of hypertension between 17.5% and 22.9% [172, 173].

The aetiology of hypertension in acromegaly is multifactorial and a number of responsible pathophysiological mechanisms have been described. Studies in both humans and animals have shown that GH has an antinatriuretic action, associated with activation of renin-angiotensin-aldosterone system [174, 175]. Administration of biosynthetic GH in healthy volunteers resulted in fluid and sodium retention, with reduction in the 24-hour urine volume and urine sodium and elevation in the plasma renin activity and aldosterone level [175]. An additional mechanism through which GH exerts its antinatriuretic action is via a direct stimulation of renal sodium reabsorption. It has been shown that GH activates the epithelial sodium channel (ENaC) in the late distal nephron, stimulating sodium reabsorption, which is independent of the renin-angiotensin-aldosterone system [176]. Normalization of the increased ENaC activity has been demonstrated following biochemical remission of acromegaly [177].

Additionally, IGF-1 has also been shown to have fluid and sodium-retaining actions. Administration of IGF-1 in healthy volunteers resulted in significant increase of the extracellular volume, without significant change in the plasma aldosterone and renin activity [178]. However, the role of IGF-1 in the regulation of arterial blood pressure is probably more complex. As mentioned previously, IGF-1 reduces peripheral vascular resistance and vascular tone [133-135]. Therefore, the balance between the opposing actions of GH and IGF-1 is what determines whether a patient with acromegaly will develop hypertension on not.

1.5.2.vi. Cerebrovascular disease

Although there are studies which have shown no difference in the incidence of stroke between patients with acromegaly and the general population [162], a recent meta-analysis has demonstrated that mortality associated with cerebrovascular disease is increased in acromegaly [179]. A number of well-recognised risk factors for stroke disease are also related to acromegaly, including arterial hypertension, diabetes and insulin resistance. Pituitary radiotherapy has been shown to increase not only the risk for cerebrovascular disease but also

the mortality associated with this [180]. However, the effect of radiotherapy on stroke incidence and mortality is independent to acromegaly, as similar outcomes have been reported in patients who had radiotherapy for other pituitary adenomas [181, 182].

1.5.2.vii. Respiratory complications

Respiratory complications in patients with acromegaly include sleep apnoea syndrome and respiratory insufficiency and are the result of a number of anatomical changes that involve the soft tissues and muscles of the craniofacial region and upper airway tract, as well as the respiratory muscles and the rib cage skeleton, leading to structural and functional alterations of the respiratory system [123]. Macroglossia, hypertrophy of the nasal structures, soft palate and laryngeal mucosa, thickening of the vocal cords and prognathism can cause obstruction of the airflow through the upper airway tract system, leading to obstructive sleep apnoea (OSA) [183]. In addition to the above, narrowing of the airways of the lower respiratory tract, derangement of the respiratory muscles and changes in the rib cage geometry (including enlargement of vertebral bodies, kyphoscoliosis of thoracic spine and elongation of the ribs) can result into stiffening of the rib cage and impaired breathing [123].

The average prevalence of OSA in patients with acromegaly has been estimated at 69%, based on a number of cross-sectional studies [184]. In contrast, reports from large National Acromegaly Registry studies suggest a lower prevalence of OSA in patients with acromegaly, between 14% and 26% [150, 161, 171]. However, these results may have been influenced by the frequency of sleep apnoea screening and therefore may have underestimated the actual prevalence of OSA in the acromegaly population. An overall increased risk of sleep apnoea in acromegaly, with an estimated hazard ratio of 11.7, has been reported, based on the results from a Danish population study [163].

A number of studies have demonstrated improvement to the sleep apnoea syndrome following either surgical or medical treatment of acromegaly [185-191]. One possible mechanism, through which this is achieved, is by reducing the tongue volume and subsequently the degree of upper airway obstruction [186, 190]. A significant reduction in the mean apnoea-

hypopnoea index (AHI), a tool used for assessing the severity of OSA, has been reported to occur as quickly as one month following pituitary surgery in patients with newly diagnosed acromegaly, with further improvement after six months, suggesting that the resolution of OSA in a time-dependent phenomenon following successful treatment of acromegaly [186]. Treatment with either somatostatin analogues or the GH-receptor antagonist, pegvisomant, has been shown to reduce the rates of OSA in a number of acromegaly cohorts [187-191]. However, OSA can persist even after biochemical remission of acromegaly in between 25% and 39% of patients [185, 192], suggesting that there are other factors, apart from disease activity, which influence the presence of sleep apnoea syndrome. BMI, which is a well-recognised risk factor for OSA in the general population, has also been shown to be implicated with the presence of sleep apnoea syndrome in patients with acromegaly. A positive correlation has been demonstrated between BMI and OSA [142, 190, 192], as well as between BMI and AHI [190]. Other risk factors identified in cohorts of patients with acromegaly include male gender [183, 192] and older age [190, 192]. No association has been demonstrated between reduction in GH/IGF-1 and improvement in the AHI in a number of studies [142, 190, 191].

Pulmonary insufficiency has been reported in 30-80% of patients with acromegaly [193]. Pulmonary function tests performed in patients with acromegaly have revealed increased lung volumes, total lung capacity (TLC), forced vital capacity (FVC) and residual volume (RV) compared with controls [186, 194, 195]. It has been proposed that the larger lung volume is due to increased alveolar size [196]. Increased airway resistance [194] and small airway obstruction, as evident by reduced peak expiratory flow (PEF) and reduced maximum expiratory flow when 75% of the forced vital capacity has been exhaled (FEF75), have also been described, suggestive of small airway disease and reduced lung elasticity in patients with acromegaly [195]. In an observational study, high resolution CT showed air trapping, airway calcifications and bronchiectasis in 60%, 40% and 35% of acromegaly cases, respectively [194]. Air trapping and small airway obstruction are thought to be due to the increasing lung volume, which is confined within the limited space of the rigid rib cage of the acromegalic

patients. In contrast, large airway obstruction has not been observed, as evident by higher values of forced expiratory volume in one second (FEV1) in patients with acromegaly [195]. Analysis of arterial blood gases has shown hypoxaemia in patients with acromegaly compared with controls, however this was clinically relevant only in 5.6% of these patients and particularly in those with elevated BMI [195]. A further cross-sectional study has shown that patients with active acromegaly have a larger volume of non-aerated or poorly aerated areas using multidetector CT for quantification of volume, which is considered to provide higher accuracy in predicting pulmonary function and assessing regional volumes in different lung compartments [197].

There are limited data regarding the effect of treatment for acromegaly on respiratory function. In a prospective study of 24 patients with active acromegaly, no change in lung volumes and respiratory muscle hypofunction were observed over a 6-month follow-up period from successful trans-sphenoidal surgery and disease remission [186]. Similarly, no difference in lung volumes and small airway obstruction was found between patients with active disease and those in remission, in a cross-sectional study [195].

Alterations in the respiratory function appear to have a significant impact on the physical performance of patients with acromegaly. Clinical studies assessing exercise capacity in patients with active acromegaly showed reduced ventilation threshold (VeT), which is a physiological marker of the ability to perform submaximal prolonged exercise, and maximum oxygen uptake (VO_{2max}) [198, 199]. These results indicate that patients with acromegaly exhibit reduced exercise capacity and have a lower threshold to develop metabolic acidosis as a result of physical activity; hyperventilation; and inability to sustain performance.

1.5.2.viii. Metabolic complications

GH is the primary anabolic hormone during stress and fasting that exerts a number of actions, affecting lipid and glucose metabolism. Acromegaly has been associated with dyslipidaemia, impaired glucose tolerance, insulin resistance and diabetes mellitus, which are well-recognised risk factors of cardiovascular morbidity and mortality.

1.5.2.viii.1. Lipid metabolism

Lipid abnormalities in acromegaly predominantly relate to hypertriglyceridaemia and low high-density lipoprotein (HDL) cholesterol, whereas total cholesterol and low-density lipoprotein (LDL) cholesterol are not dissimilar to the general population [150, 200], with some studies also suggesting lower levels of total and LDL cholesterol compared with healthy controls [201]. Hypertriglyceridaemia has been reported in 33-40% of patients with acromegaly, while the prevalence of low HDL-cholesterol has been estimated at 39-47% [202, 203].

Studies of administration of GH in normal individuals have shown enhancement of lipolysis and lipid oxidation leading to increased release of serum free fatty acids (FFAs), 3-hydroxybutyrate and glycerol levels from the adipose tissue [204, 205]. This is in-keeping with observational studies showing reduced amount of body fat in patients with acromegaly [206], while GH-deficient adults have increased fat mass and reduced lean body mass [207]. GH has also been shown to inhibit the activity of the lipoprotein lipase in the adipose tissue [208]. Lipoprotein lipase is an enzyme that catalyses the hydrolysis of triacylglycerols in chylomicrons and very low density lipoproteins and its inhibition may lead to reduced uptake of triglycerides by the adipose tissue, contributing to the higher triglycerides concentration [209]. Another mechanism for dyslipidaemia in patients with acromegaly is via GH-induced adipose tissue inflammation, due an increase in the expression of adipokines, such as visfatin and interleukin-6 [210, 211].

Although IGF-1 mediates the actions of GH in many peripheral tissues in general, it appears that the two hormones have opposing effects on the lipid metabolism. Therefore, in contrast to the actions of GH, IGF-1 reduces FFAs levels in the serum by increasing the uptake of FFAs by adipocytes and hepatocytes [212]. Additionally, IGF-1 has been shown to promote lipogenesis, although this latter function appears to be of a lesser significance in the overall FFA homeostasis [213].

Treatment of acromegaly with either somatostatin analogues or trans-sphenoidal surgery, as first line therapeutic intervention, has been shown to improve dyslipidaemia in the majority,

but not in all, cases. In a retrospective study of 231 patients with acromegaly, 12.4% and 24.8% of patients who achieved biochemical remission with either medical or surgical treatment continued to have hypertriglyceridaemia and low HDL-cholesterol respectively, after a 12-month period of follow-up [212].

1.5.2.viii.2. Glucose metabolism

Acromegaly is associated with insulin resistance, which is the result of a number of different pathophysiological processes mediated by GH. The main mechanism for the development of insulin resistance is the impaired insulin-stimulated glucose uptake due to the increase in FFAs, which causes a reduction in the expression of glucose transporters 1 and 4 in the adipose tissue [214]. Additionally, GH excess inhibits intracellular insulin signaling by blocking insulin-receptor substrate-1 and phosphoinositide 3-kinase, which are implicated in the glucose transport in the adipose tissue and muscle [215]. GH also promotes gluconeogenesis in the liver, leading to increased glucose synthesis and thus contributing further to impaired glucose tolerance [216]. The reduction in the fat mass in patients with acromegaly and the GH-induced adipose tissue inflammation, described previously, consist an additional mechanism that results in insulin resistance [210, 211]. Finally, impaired function of the pancreatic β-cells, as measured by homeostasis model assessment (HOMA), has been demonstrated in some patients with acromegaly. It has also been shown that in patients with β-cell dysfunction, impaired glucose metabolism persists even after successful treatment of acromegaly [217].

In contrast to the above-mentioned adverse effects of GH on the glucose metabolism, GH promotes insulin synthesis and secretion [218]. Additionally, IGF-1 has a positive impact on glucose homeostasis and insulin resistance by increasing the muscle glucose uptake [212]. However, chronic GH excess leads to insulin resistance in the liver, adipose tissue and muscle, by mechanisms previously described, which eventually overcomes any beneficial effect of IGF-1 on glucose metabolism.

Diabetes mellitus (DM) has been reported in 16-56% of patients with acromegaly based on a literature review [214]. This high variability in the prevalence of diabetes is thought to be related to differences in the ethnicity amongst clinical studies, as well as differences in the criteria used for the diagnosis of diabetes (oral glucose tolerance test, fasting glucose or HbA1c). Overall, the prevalence of DM in acromegaly has been found to be higher compared with the general population and in some studies, after including patients with impaired fasting glucose and impaired glucose tolerance, only 22% of patients with acromegaly were found to have entirely normal glucose metabolism [219].

Similar to the general population, DM in acromegaly has been associated with obesity [219, 220], older age [219-221] and family history of diabetes [220]. Unlike the general population, gender-related differences in the prevalence of metabolic syndrome have been in reported patients with active acromegaly, with female patients demonstrating a more adverse metabolic profile characterized by higher fasting insulin levels, increased insulin resistance and elevated visceral adiposity compared with male patients [203]. Factors specific to acromegaly, which have been shown to be associated with DM, include longer duration of active disease and presence of a pituitary macroadenoma [221, 222]. The biochemical markers of acromegaly, GH and IGF-1, have been associated with the presence of DM in some [219-221], but not all studies [222].

There are no specific guidelines for the management of diabetes secondary to acromegaly. Therapeutic strategies include lifestyle modifications (diet and exercise), oral medications similar to those used in the general population with type 2 diabetes and insulin [214]. The severity of diabetes in patients with acromegaly varies significantly and although a large proportion of patients have mild diabetes, there have been cases in the literature of patients who presented with diabetic ketoacidosis as the first manifestation of acromegaly [223].

The different therapeutic interventions for the management of acromegaly exert different effects on glucose metabolism. Disease remission achieved by trans-sphenoidal surgery has been shown to improve insulin resistance and reduce prevalence of DM [202]. Normalization of glucose metabolism in 23-58% of patients with pre-existing diabetes or impaired glucose

tolerance has been demonstrated within two months after trans-sphenoidal surgery in some series [217, 224]. There are a number of clinical studies, in which the effects of first and second-generation somatostatin analogues on glucose homeostasis have been explored. Somatostatin analogues (SSAs) act by binding and activating the somatostatin receptor (SSTR); and 5 different subtypes of SSTRs (SSTR1-5) have been reported. First generation SSAs (Octreotide LAR and Lanreotide Autogel) bind with higher affinity for the SSTR2 compared to SSTR5, whereas the second generation SSA, pasireotide, has a 40-fold higher affinity for SSTR5 and 2-fold lower affinity for SSTR2 compared with the first generation SSAs [225]. Additionally, SSTRs can also been found in the pancreatic α-cells and β-cells, with higher levels of SSTR2 expressed in α-cells and higher levels of SSTR5 expressed in β-cells [226]. Therefore, SSAs can affect the secretion of glucagon and insulin, resulting in changes in glucose metabolism.

The results of a meta-analysis showed that first generation SSAs led to a reduction in insulin levels, without causing significant alterations to fasting glucose levels or HbA1c, suggesting that this class of drugs exerts only a mild effect on glucose homeostasis, without clinically significant adverse outcomes [227]. A more recent prospective study has shown that the use of high dose Lanreotide Autogel as first-line treatment with patients with active acromegaly led to a significant reduction in HbA1c in patients with pre-existing diabetes after a 12-month follow-up period, however no significant change was found in the overall study population or in the subgroup of acromegalic patients without pre-existing diabetes [228]. In contrast, pasireotide, the only second-generation SSA available, due to its higher affinity to the SSTR5, causes a much more profound inhibition of insulin secretion, with a significantly more modest effect on the inhibition of glucagon [229]. Two phase III clinical trials have shown high rates of hyperglycaemia-related adverse events in up to 67% of patients treated with pasireotide [230, 231], while diabetes was reported in up to 26% of those patients [231]. Notably, 25% patients were required to commence medical therapy for diabetes, whilst on pasireotide [232]. Patients with fasting glucose >100 mg/dL (or >5.6 mmol/L) before the start of pasireotide treatment were found to be at higher risk of developing hyperglycaemia, associated with the

treatment [232]. However, it appears that the diabetes associated with pasireotide may be reversible with treatment discontinuation, and that mechanistically it differs from other forms of diabetes.

Treatment with pegvisomant has been shown to have a favourable impact on glucose homeostasis, improving fasting glucose and HbA1c, with the greatest reduction in HbA1c observed in acromegalic patients with pre-existent diabetes [233]. Reduction in the insulin levels with pegvisomant has also been reported, possibly due to the lower glucose levels and HOMA-index [234]. Dopamine agonists have also been used in the medical management of acromegaly. In a small study, bromocriptine was found to improve fasting glucose levels and glucose tolerance [235], however there are no data regarding outcomes on diabetes and insulin resistance with the newer dopamine agonists (cabergoline and quinagolide).

1.5.2.ix. Bone Metabolism

GH and IGF-1 play an important role in bone metabolism and physiologically are considered to have an anabolic effect on the skeleton, promoting longitudinal bone growth, bone maturation and acquisition of bone mass in the pre-pubertal phase, while in adulthood they are responsible for maintaining bone mass [236, 237]. However, emerging evidence indicates that growth hormone excess in acromegaly has adverse outcomes in bone turnover and bone microarchitecture, increasing the risk of fragility fractures [218].

1.5.2.ix.1. Bone turnover and calcium metabolism

Increased bone turnover has been found in patients with acromegaly, with higher bone formation and bone resorption biochemical markers compared with controls, however the increase in the bone resorption is disproportionate to the increase in bone formation leading to skeletal fragility [239]. GH enhances proliferation, maturation and function of osteoblasts both via a direct effect and also via the actions of IGF-1 [240-243]. Additionally, IGF-1 promotes transcription of type I collagen and reduces collagenase-3, which is a collage-degrading protease [244]. The synergistic impact of the above actions of GH/IGF-1 produces

an anabolic effect, which leads to increased bone formation. The mechanisms responsible for increased bone resorption in acromegaly have been less well characterized. One potential hypothesis includes an IGF-1 mediated increased formation and activation of osteoclasts, which express IGF-1 receptors [245].

Patients with active acromegaly often have mild biochemical abnormalities of bone profile parameters, such as hypercalcaemia, hypercalciuria and hyperphosphataemia [246, 247]. The changes in the calcium/phosphate homeostasis are considered to be independent of PTH [246]. The hypercalciuria has been associated with increased calcium absorption from the gut due to GH-mediated activation of vitamin D [248], as well as with bone turnover and has been considered as a marker of skeletal fragility [246]. Although GH stimulates renal activation of vitamin D to 1,25 dihydroxyvitamin D [248], hypovitaminosis D has also been described in patients with acromegaly and has been attributed to reduced bioavailability of vitamin D as a result of increased vitamin D binding protein [249].

1.5.2.ix.2. Bone mineral density

Assessment of bone mineral density (BMD) by dual-energy x-ray absorptiometry (DXA) is unreliable in patients with acromegaly, as there are several limitations in the interpretation of BMD results in this group of patients. Osteophyte formation and facet joint hypertrophy, which are common findings in acromegaly, may lead to a falsely elevated BMD in the lumbar spine [250]. Additionally, bone enlargement, as a result of GH excess, can affect BMD as estimated by DXA [251]. Another caveat in the interpretation of DXA results in patients with acromegaly is that GH has a different effect on cortical and trabecular bone. GH promotes periosteal ossification of cortical bones and therefore increases cortical BMD, however it weakens trabecular bone microarchitecture [243]. Therefore, the overall BMD depends on the distribution of cortical and trabecular bone at the different skeletal sites and as a consequence, DXA, which is unable to distinguish between the two different bone compartments, cannot provide qualitative data on bone microarchitecture or be used as a reliable tool to assess fracture risk in patients with acromegaly. BMD in acromegaly is often reported normal or

elevated. A meta-analysis of 1935 patients with acromegaly showed increased BMD at the femoral neck compared with controls, whereas no significant difference was found in the lumbar spine BMD [238]. As expected, BMD has not been found to be predictive of vertebral fractures in patients with acromegaly [252] and as a result alternative techniques have been applied to assess bone microarchitecture, including high-resolution peripheral quantitative CT, trabecular bone score and impact microindentation.

High resolution peripheral quantitative CT (HR-pQCT) is able to provide data on three-dimensional, volumetric BMD, as well as distinguish between cortical and trabecular bone, and studies using this modality have shown reduction in the trabecular volumetric BMD in patients with acromegaly compared with controls irrespective of disease activity status [253, 254]. Regarding cortical volumetric BMD, some studies suggest that acromegaly does not have any negative impact on cortical bone [253], whereas other studies have shown reduced cortical volumetric BMD in patients with acromegaly [254]. Trabecular bone score (TBS) is a novel tool, developed based on lumbar spine DXA images, that captures the mean rate of pixel gray-level variations in the DXA images and evaluates bone microarchitecture, with higher scores indicating a more favourable bone structure [255]. In a cross-sectional study of 33 patients with active acromegaly, lumbar spine TBS was found to be reduced compared with age, gender and BMI-matched controls, despite no difference in the BMD between the two groups [256]. Impact microidentification is a novel technique, used for the measurement of bone mechanical strength in vivo, which involves the insertion of a test probe through the skin to the bone surface of the midshaft of the tibia. This technique creates microfractures and measures the resistance of the bone tissue to fracture [257]. When applied to 48 patients with acromegaly in remission and 44 controls, patients with acromegaly had significantly lower bone material strength index than controls, despite comparable BMD in the two groups [258].

1.5.2.ix.3. Fragility fractures

High rate of radiological vertebral fractures has been reported in patients with acromegaly, with the results of a meta-analysis suggesting a combined prevalence of 38% based on the

results of 10 studies [238]. Vertebral fractures were more commonly found in the thoracic spine than in the lumbar spine and were mostly anterior wedge fractures [252]. The majority of the vertebral fractures were mild in severity, defined as less than 25% reduction in vertebral height [252]. Overall patients with acromegaly are considered to have a significantly higher risk for radiological vertebral fractures compared with controls, with an odds ratio of 8.26 [238]. Patients with active disease were found to have a 3-fold higher risk for vertebral fractures compared with patients in remission [238]. Other factors identified to be associated with higher risk of vertebral fractures in acromegaly include male gender (odds ratio 2.19) and hypogonadism (odd ratio 1.68) [238]. Notably, no difference in lumbar BMD was found between acromegalic patients with and without vertebral fractures [238]. However, patients with vertebral fractures were shown to have abnormal trabecular bone properties, with lower bone volume/trabecular volume ratio, reduced trabecular thickness and greater mean trabecular separation, compared with controls, as evaluated by high resolution cone beam tomography [259].

Diabetes mellitus is another potential risk factor for osteoporotic fractures. This is already well-established in the general population [260] and DM appears to also have a similar negative impact in acromegaly, particularly in patients with disease remission. Higher prevalence of DM was found in patients with acromegaly who had vertebral fractures compared with those who did not. Although active acromegaly was associated with increased prevalence of vertebral fractures irrespective of the presence of DM, patients with disease remission had significantly higher prevalence of vertebral fractures in the context of co-existing diabetes [261]. The role of genetic factors in the risk of vertebral fractures has also been explored in some clinical studies, however conflicting data have been generated. Deletion of exon-3 of the GH receptor (d3GHR), which increases the receptor sensitivity to GH and enhances the actions of GH, was associated with increased prevalence of vertebral fractures in patients with both active disease and disease remission, compared with patients who carried the full-length isoform of the GH receptor [262]. In contrast, in another study, presence of the d3GHR polymorphism was associated with increased prevalence of

arthropathy, colonic polyps and dolichocolon, but not with reduced BMD or vertebral fractures, although this study was conducted in a cohort of patients with long-term remission of acromegaly [263], rather than a mixed population of patients with active and controlled disease as in the aforementioned study by Mormando et al [262].

Successful treatment of acromegaly and biochemical disease control does not reduce future risk for vertebral fractures, despite reports, which suggest that bone turnover markers decline and BMD improves with treatment [246, 264]. In a prospective study on 49 patients with controlled acromegaly, a 20% progression in vertebral fractures compared with baseline was noted over a 2.5-year follow-up period [265]. Male patients, as well as patients with previous vertebral fractures were at a higher risk for new incidence of vertebral fractures, suggesting that abnormal bone microarchitecture persists even after disease remission and irrespective of BMD [265]. Currently, there are no available data regarding the use of antiresporptive agents (bisphosphonates, denosumab) and the anabolic agent teriparatide (recombinant human parathyroid hormone), which are the mainstay therapies for the management of osteoporosis in the general population, in patients with acromegaly. Additionally, it remains unknown whether these drugs impact on the abnormal bone qualities and fragility fracture risk in this particular group of patients [239].

1.5.2.x. Neoplastic complications

The potential involvement of GH to the process of tumorigenesis was first conceptualised in the 1950s, when it was noted that cancer remission was induced following hypophysectomy in female patients with metastatic breast cancer [266]. Since then there has been a plethora of studies, both in vitro and in vivo using animal models, in which the GH/IGF-1 axis was genetically modified, to investigate the role of GH and IGF-1 in tumour growth and development. However, how these outcomes translate into cancer risk in clinical practice in patients with acromegaly remains debatable.

1.5.2.x.1. Effects of GH and IGF-1 on tumour biology

Expression of GH/IGF-1 and their corresponding receptors has been shown in different types of tissues, including colorectal, breast, prostate, thyroid, endometrial, renal, pancreas and brain tumour cells [267-269]. Autocrine production of human GH by mammary carcinoma cells was shown to have a mitotic effect, promoting cell proliferation [270]. GH also enhances angiogenesis and lymphangiogenesis, promotes cell survival and cell migration leading to increasing tumour invasiveness and metastasis [271-274]. Additionally, IGF-1 and IGF-2 induce the expression of hypoxia-inducible factor 1 in cultured cells, which activates the transcription of vascular endothelial growth factor and promotes angiogenesis, an important step in the tumour progression process [275]. The regulation of cancer physiology by the GH/IGF-1 axis is complicated by the role of IGF binding proteins, which have been shown to induce apoptosis and inhibition of tumour growth, when overexpressed in animal models, attenuating the carcinogenic effect of IGF-1 [276].

Further studies in colon cells have shown that GH may also promote tumour growth by attenuating the expression of p53, a tumour suppression protein, as well as the expression of the tumour suppressor adenomatous polyposis coli (APC), which are both implicated in the pathophysiology of colorectal cancer [277]. There is also evidence that autocrine human GH increases the resistance of breast and endometrial carcinoma cells to ionizing radiation and chemotherapy agents, suggesting that inhibiting the autocrine actions of human GH could be a potential target in cancer therapy [278-280].

Regarding human studies, a number of meta-analyses have identified a weak, however, positive association between adult height and a variety of cancers, including breast, colorectal, thyroid, renal and pancreas cancer [281-283]. Additionally, IGF-1 values in the highest quartiles have been associated with increased risk of malignancy in the colon, breast and prostate [284, 285]. In keeping with the above observations are epidemiological data from individuals with Laron syndrome, a rare condition characterized by short stature due to a mutation in the GH receptor, resulting in GH insensitivity and IGF-1 deficiency. This particular group of patients has a significantly reduced incidence of malignancies compared

with their unaffected relatives, whose risk of developing cancer is similar to the general population [286]. In contrast, no significant increase in the incidence of all forms of cancer was found in children with GH deficiency, treated with recombinant human GH at therapeutic doses [276]. There are less data about the safety of GH replacement in adults with GH deficiency, however results from the KIMS database (an international metabolic database by Pfizer with data from over 6,000 adults treated with GH followed up prospectively) suggest that the incidence of de novo cancer in GH-treated adults is similar to the general population [287].

The GH/IHF-1 axis as a potential target for cancer treatment has also received significant amount of attention. The GH receptor antagonist, pegvisomant, has been shown to have anti-proliferative properties, inhibiting tumour growth both as monotherapy [288-290], but also synergistically with radiotherapy [291] and chemotherapy [292]. Inhibition of the GH receptor also enhanced transcription of the tumour suppressor p53 protein and APC [277]. Other targets in cancer therapy include blockade of the GHRH and the IGF-1 and IGF-2 receptors. Positive results in terms of tumour growth inhibition have been produced by the use of GHRH antagonists [293] and neutralizing antibodies against the IGF-1 and IGF-2 receptors [294, 295] in various cancer cell lines.

1.5.2.x.2. Cancer incidence in acromegaly

There is inconsistency in the data amongst different studies regarding the risk of malignancy in patients with acromegaly. Standardised incidence ratios (SIR) for the incidence of cancer in acromegaly from large multi-centre studies vary significantly, with some studies showing increased SIR, others showing similar or even lower SIR compared with the cancer incidence in the general population. A modest increase in the risk of cancer with a SIR between 1.4 and 1.5 has been reported by three nation-wide studies; two performed in Scandinavia [296, 297] and one in Italy [298]. The SIR of 1.34 in the French Acromegaly Registry study was not statistically significant [150], whereas the results of the German study suggested a trend towards lower cancer incidence in patients with acromegaly compared with the general

population (SIR 0.75), although this observation did not reach the level of statistical significance [299]. In contrast, the results of a large multi-centre UK study from 1239 patients with acromegaly followed up for just over 13 years showed a significantly lower cancer incidence due to all malignanies in comparison with the general population, with a SIR of 0.76 [300]. A recent meta-analysis of 23 studies, showed a combined SIR of 1.5 for overall cancer incidence, however a significant heterogeneity was found amonst the studies included [301]. When small-size single-centre studies were excluded from the analysis, the SIR was calculated at 1.2 amongst 6 multi-centre studies, which was not statistically significant and at 1.4 between two population studies, which was significantly higher than in the general population, indicating that acromegaly is associated with only a modest increase in the cancer incidence [301].

Regarding incidence rates of malignancy specific to different anatomical sites, the results of the aforementioned meta-analysis showed increased SIR for colorectal, thyroid, gastric, breast and urinary tract cancer, with significant differences in the results between single and multi-centre studies [300]. Notably, data from three multi-centre studies in Germany, Denmark and the UK did not reveal increased cancer incidence at any anatomical site [299-301]. The highest SIR was noted for thyroid cancer (combined SIR 9.2), whereas no difference was observed in the incidence of haematological malignancies, lung and prostate cancer between patients with acromegaly and the general population [301]. Interestingly, no association has been demonstrated between GH/IGF-1 levels and cancer incidence across a number of different studies [150, 298, 299].

Overall, the controversy regarding the potential link between acromegaly and cancer risk continues to exist, however emerging data suggest that although acromegaly may be associated with a modest increase in cancer incidence, GH excess is not a serious cancer risk. Finally, consideration needs to be given to the changes in life expentancy in patients with acromegaly when interpreting results about cancer incidence. With the introduction of modern therepies in the last two decades, achieving biochemical disease control has become more common and survival of patients with acromegaly is not dissimilar to the general

population [179]. Therefore studies performed in older eras, prior to the recent advancements in the management of acromegaly, may have underestimated cancer incidence, as a results of increased mortality from other complications of acromegaly, such as cardiovascular disease, occurring at an early stage in the patient's life, when malignancy is not likely to have developed.

1.5.2.x.3. Colonic polyps and cancer

The results of a meta-analysis have shown increased incidence of colon cancer in patients with acromegaly, with a SIR of 2.6. However, there still remains controversy regarding the potential association between acromegaly and increased risk for colon cancer, based on the results from individual studies, with some studies being in favour of this association [296, 302], while other studies do not demonstrate increased incidence of colon cancer in patients with acromegaly compared with the general population [163, 298, 300, 303].

In contrast to colon cancer, the association between acromegaly and colonic polyps has been well-established. There is a wide variation in the prevalence of colonic polyps in patients with acromegaly reported amongst different studies, with the rate ranging between 7% and 76% [160]. According to a meta-analysis in 2008, the pooled prevalence of colonic adenomas and hyperplastic colonic polyps in acromegaly was calculated at 23.2% and 22.3% respectively. Additionally, patients with acromegaly had a higher risk of harbouring a colonic polyp compared with controls with a pooled odds ratio of 2.5 for colonic adenomas and 3.6 for hyperplastic colonic polyps [304].

IGF-1, but not GH levels has been found to be associated with the presence of colonic polyps at the time of first colonoscopy [160, 305, 306]. Patients with colonic polyps at baseline colonoscopy and with persistently elevated IGF-1, suggestive of on-going disease activity, were found to be at increased risk of developing new colonic lesions on subsequent colonoscopies [306, 307]. Conversely, in the absence of colonic adenomas at initial screening, colonic polyps are unlikely to develop during future surveillance irrespective of the biochemical disease control [307]. A normal colonoscopy at baseline was associated with

78% chance of a normal second colonoscopy, while patients with a normal second colonoscopy had 81% chance of a normal examination the third time [306].

The age, in patients with acromegaly, at which screening for colonic lesions should be initiated has been an area of conflicts. Some authors, including the British Society of Gastroenterology, have proposed the age of 40 years, as a cut-off for initiating the colonoscopy surveillance programme [306, 308, 309]. However, a large multi-centre study from Italy reported presence of colonic neoplasia in 19.3% of patients with acromegaly younger than 40 years of age, which was significantly higher than the rate observed in the control group of a similar age [310]. In view of this, the American Endocrine Society recommends colonic screening in all patients with acromegaly at the time of diagnosis [311]. Future surveillance following treatment of acromegaly depends on the findings of the initial screening as well as the disease activity status. Therefore, colonoscopy is recommended every 5 years in patients who were found to have a polyp at baseline screening or in those with persistently elevated IGF-1 and every 10 years in patients who had a normal baseline colonoscopy and IGF-1 levels within the age-specific reference range [311].

Other colon abonormalities that have been observed in patients with acromegaly include increased bowel length, tortuous bowel and diverticular disease [305], which can impose practical difficulties during colonoscopy and prolong the time required to complete the examination [312].

1.5.2.x.4. Thyroid nodules and cancer

Acromegaly is associated with nodular thyroid disease and increased thyroid volume [313-316]. Human thyroid follicular cells express IGF-1 receptors and under the stimulatory effect of GH they can also synthesize IGF-1, which acts in an autocrine fashion to promote thyroid cell proliferation [317]. Studies have shown a positive correlation between thyroid volume and GH/IGF-1 levels [313, 314], as well as between presence nodular thyroid and duration of active acromegaly [313, 315].

A meta-analysis of 11 studies showed that patients with acromegaly had a pooled prevalence of 59.2% for thyroid nodules (with the rate varying between 43.2% and 75.6% amongst the different studies) and a pooled prevalence of 4.3% for thyroid cancer (with the rate varying between 0.8%-11.8% amongst different studies) [318]. A separate meta-analysis of 5 case-control studies, revealed increased risk for thyroid nodules and thyroid cancer in patients with acromegaly compared with controls, with the odds ratios (OR) being 3.6 and 7.9 respectively [318]. The risk of malignancy in acromegalic patients with nodular thyroid disease was calculated at 8.7%, which is not dissimilar to the risk in the general population [318].

Results from acromegaly registry-based studies (which were not included in the aforementioned meta-anlysis) have mostly shown lower rates of thyroid cancer compared with case-control studies. In a multicentre UK study only one case of thyroid cancer was found in 1239 patients with acromegaly [300]. Similarly, in a German study 3 cases of thyroid cancer were reported among 446 cases of acromegaly and the SIR for thyroid cancer was not significantly increased [299]. In contrast, significantly increased SIR for thyroid cancer at 3.6 was found in a multicentre Italian study, in which 13 cases of thyroid cancer were identified among 1512 patients with acromegaly [298]. An even higher SIR of 13.4 was reported based on the results of the Finnish acromegaly registry, with 6 cases of thyroid cancer in 313 patients with acromegaly [297].

The discrepancy in the results between case-contol and registry-based studies could be due data inaccuracies in the registries or due to variations in clinical practice in terms of screening for thyroid cancer. Another element that adds to the controversy about the potential increased risk of thyroid cancer in acromegaly, is the uncertainty regarding the actual prevalence of thyroid cancer in the general population. Thyroid cancer often remains asymptomatic and routine screening is not recommended. Therefore it is possible that thyroid cancer is underdiagnosed in the general population, as it may be in the acromegaly registry-based studies. Similar to the general population, the most common histological type of thyroid cancer in patients with acromegaly is differentiated papillary thyroid carcinoma [316, 319-321].

The American Endocrine Society advocates that a thyroid ultrasound should be performed in case of palpable thyroid nodularity in patients with acromegaly [311]. In the remaining cases, where there is no palpable goitre clinical examination of the thyroid gland annualy has been recommended [160]. Fine needle aspiration of thyroid nodules in patients with acromegaly should be guided by the same principles as in the general population [160].

1.6. Diagnosis of acromegaly and relevant investigations

Diagnosis of acromegaly is clinical, based on the signs of the condition, including body disfigurement, acral enlargement, skeletal changes and soft tissue swelling, which are described in detail in the section of "Clinical Features". However, biochemical confirmation of GH excess is pivotal. This requires evidence of autonomous GH hypersecretion, as well as elevated IGF-1, which confers the biologic effect of GH excess to the peripheral tissues and organs [20]. Clinical diagnosis is often delayed as symptoms of acromegaly develop in an insidious way and therefore can remain unnoticed by the patients for a number of years. A delay of 6.6-10.2 years has been estimated between the time of onset of symptoms and diagnosis of acromegaly [105].

When acromegaly is suspected based on clinical grounds, measurement of IGF-1 has been proposed as the initial screening test [311]. This is because IGF-1 has a relatively long half-life of 15 hours and therefore serum levels remain overall stable [322]. In contrast, GH is secreted in a pulsatile way and GH levels may flunctutate from being undetectable to up to 30 mcg/L in a healthy individual without a pituitary pathology [323]. Therefore random GH measurements are not recommended as a screening test to diagnose acromegaly [311]. IGF-1 as a screening test for acromegaly is also indicated in patients with an incidental finding of a pituitary mass, as well as in patients without the typical clinical manifestations of acromegaly, but with other associated conditions such as sleep apnoea, diabetes mellitus, hypertension, carpal tunnel syndrome, hyperhidrosis, severe arthropathy. However, no specific guidelines exist for the latter occasion and clinic judgement is required, considering that these comorbidities are frequently encountered in the general population [311]. When interpreting

IGF-1 results, special consideration needs to be given to certain patient groups. Pregnancy and late adolescence are associated with high IGF-1 levels, which may cause false positive results [20]. Conversely, patients with liver disease, renal failure, malnutrition and those on oestrogen treatment may have low IGF-1 levels [20, 324, 325]. Nevertheless, in principle, a normal IGF-1 value is considered that excludes the diagnosis of acromegaly [311].

Patients with raised IGF-1 levels should undergo an oral glucose tolerance test (OGTT), which is the gold-standard test for the diagnosis of acromegaly. Inability to suppress GH to <1 mcg/L within two hours after a 75g oral glucose load has been generally accepted as the preferred dynamic test to demonstrate autonomous GH secretion [311]. However, depending on the assay used for measuring GH, the cut-off of 1 mcg/L may not be highly sensitive and may produce a number of false negative results, as elevated IGF-1 levels with nadir GH of <1 mcg/L on the OGTT can be found in up 50% of patients with acromegaly, when GH is measured with highly sensitive GH assays [326]. The first GH assays were polyclonal competitive radio-immunoassays (RIAs) and were of low sensitivity and therefore a cut-off of <1 mcg/L in the nadir GH during an OGTT may have been appropriate in those cases. However, over the years more sensitive assays have been developed including the non-competitive two-site antibody radioimmunometric assays (IRMAs) and the non-isotopic two-site antibody immunochemiluminescent assays, which can detect GH levels as low as 0.05 mcg/L. Therefore, when using these highly sensitive GH assays, a nadir GH of greater than 0.3 mcg/L during the OGTT has been found to be more sensitive for diagnosing acromegaly and also for distinguishing between patients with active disease and disease remission following treatment of acromegaly [327, 328]. However, GH levels may fail to become suppressed in patients with severe liver disease, renal impairment, poorly controlled diabetes and malnutrition [20].

Once biochemical confirmation of acromegaly is established, further investigations are required to localize the source of the autonomous GH hypersecretion. As more than 95% of cases acromegaly are due to a benign pituitary adenoma, imaging of the pituitary gland is the next step in the diagnostic process. Magnetic resonance imaging (MRI) with administration of

contrast is the preferred imaging modality to visualise the pituitary gland. When MRI scan is contraindicated, a computed tomography (CT) scan can be used instead. Pituitary adenomas associated with acromegaly are macroadenomas (>10 mm in size) in up to 77% of patients at the time of diagnosis [329]. Pituitary MRI is usually performed with 2mm-slices, which allows the diagnosis of microadenomas that are at least 2mm in diameter. Apart from the size of the adenoma, pituitary MRI also helps to assess the invasiveness of the tumour. Lateral extension of the pituitary tumour to the cavernous sinus is often associated with incomplete surgical resection; invasion into the sphenoid bone may lead to cerebrospinal fluid leak and rhinorrhoea; whereas superior extension into the suprasellar cistern may abut the optic chiasm, causing visual field defects. Formal visual field assessment is required in those cases when the pituitary tumour makes contact with the optic pathway [311]. In case of optic nerve compression and visual field compromise, urgent operation is required for tumour debulking at least, if no complete resection is possible.

When there is biochemical evidence of GH hypersecretion, but no adenoma is identified on pituitary imaging or alternatively presence of pituitary hyperplasia is reported, the possibility of an ectopic (extra-pituitary) source of acromegaly should be considered. Ectopic acromegaly is more commonly related to GHRH-secreting tumours and measurement of serum GHRH can help to reach this diagnosis. Imaging of the thorax and abdomen with MRI or CT scan or somatostatin receptor scintigraphy (i.e. Octreotide scan) is required in order to localise tumours associated with ectopic acromegaly [43]. Presence of symptoms which are not expected in patients with acromegaly such as respiratory wheeze, dyspnoea, flushing, peptic ulcers may occasionally help diagnosing non-pituitary tumours which could be associated with GH excess [43].

Functional assessment of the pituitary gland for the integrity of the remaining anterior pituitary hormone axes is also required. Approximately 25% of GH-secreting pituitary adenomas can co-secrete prolactin, which is due to either adenomas consisting of a mixed population of somatoroph and lactrotroph cells producing GH and prolactin respectively [24, 25] or due to adenomas originating from a single mammosomatotroph stem cell and therefore

containing a monomorphic population of cells that co-secrete GH and prolactin [26]. In some other cases, hyperprolactinaemia is due to the disruption of the normal lactotroph inhibition, which is mediated by dopaminergic signals transmitted from the hypothalamus to the anterior pituitary via the stalk. This "stalk-effect" has been observed in some cases of pituitary macroadenomas, in which the pituitary tumour creates a pressure-effect on the stalk and reduces the dopaminergic inhibition on the lactotroph cells. Hyperprolactinaemia is a well-recognised cause of hypogonadotrophic hypogonadism [330] and this has also been observed in patients with acromegaly [331, 332]. Hypogonadism has been reported in up to 72% of acromegalic patients with hyperprolactinaemia at the time of diagnosis [331].

Hypopituitarism may be present at the time of diagnosis of acromegaly and is due to compression of the normal pituitary tissue by the adenoma and therefore is usually encountered in the context of a macroadenoma. Hypogonadism, which usually manifests with amenorrhoea in pre-menopausal female patients and erectile dysfunction/reduced libido in male patients, is commonly present at diagnosis of acromegaly, found in up to 53% of patients, with higher frequency in women than men [331]. Although hyperprolactinaemia is an important contributing factor to hypogonadism, as mentioned previously, it can also be present in patients with normoprolactinaemia, as well as patients with microadenomas [331]. An observational study showed that 55% of hypogonadal patients with acromegaly had normal prolactin levels. Additionally, 25% of patients with a microadenoma had hypogonadism despite normal prolactin levels [331], which suggests that mechanisms other than tumour mass effect and hypreprolactaemia may also contribute to the development of hypogonadism in acromegaly. Secondary adrenal insufficiency and central hypothyroidism have been reported in up to 20% and 9% of patients with acromegaly respectively [123, 333]. Additionally, reduced 24-hour TSH secretion has been found in patients with acromegaly, due to lower basal and pulsatile TSH secretion, however this did not appear to have a significant impact on the free T4 levels [334].

1.7. Treatment of acromegaly

1.7.1. Treatment Goals

An effective therapeutic strategy in the management of acromegaly should aim to normalise GH and IGF-1 values and reverse the excess morbidity and mortality associated with this condition. The specific goals of acromegaly treatment include:

(i) Eliminating autonomous GH hypersecretion and normalising IGF-1. The biochemical target, which has been accepted to define remission of acromegaly, consists of GH of less than 1 mcg/L (either in a random blood sample or during a 2-hour OGGT) and an IGF-1 value within the age-specific reference range [311]. These biochemical cut-offs are based on epidemiological data which suggest that excess mortality in patients with acromegaly is restored to the expected of the general population when GH <1 mcg/L and normal IGF-1 are achieved [335].

(ii) Selective resection or reduction of the volume of the pituitary tumour, while preserving normal pituitary tissue. In patients with pituitary macroadenomas with significant suprasellar extension and optic chiasm compromise, surgical resection or debulking of the tumour also aims to reverse any visual field deficits and prevent further visual defects.

(iii) Preventing hypopituitarism and preserving normal anterior pituitary hormone function and particularly preservation of the adrenal, gonadal and thyroid axes. Hypopituitarism itself is also associated with increased mortality [336] and if present patients should be commenced on appropriate replacement therapy of the deficient pituitary hormone axes.

(iv) Reducing the morbidity associated with acromegaly by improving symptoms and signs of acromegaly (i.e. headaches, hyperhidrosis, soft tissue swelling, nerve entrapment syndromes) and simultaneously preventing systemic complications related to long-term GH excess (i.e. diabetes mellitus, hypertension, cardiomyopathy, sleep apnoea syndrome, arthropathy).

(v) Preventing disease recurrence, either biochemical or anatomical. Therefore, long-term follow-up of patients with acromegaly is required for early detection of recurrence. In these cases additional treatment or combination of different treatment modalities is often needed to prevent further adverse effects of chronic GH hypersecretion.

Treatment of acromegaly comprises of one or a combination of the following: surgery, radiotherapy and medical therapy. Successful treatment of acromegaly, achieving all the goals listed above is often challenging. Additional consideration needs to be given to the potential side effects and complications related to each of the therapeutic interventions.

1.7.2. Surgical Management

Surgical resection of the GH-secreting pituitary adenoma via trans-sphenoidal approach, using either a microscopic or endoscopic technique, is the first-line treatment in patients with acromegaly [311]. There are advantages and disadvantages associated with each of these two surgical techniques. The operative microscope allows for a single, unobstructed view field and provided 3-dimensional stereotactic images [337]. It also allows for better control of intra-operative bleeding and preserves the posterior one third of the nasal midline structures, which may explain the fewer self-reported sinusitis and alterations in taste and smell in patients who have undergone microscopic trsnssphenoidal surgery (TSS) compared with the endocscopic TSS [338]. As the microscopic technique was developed first, more neurosurgeons may be more familiar with this. In contrast the endoscope provided more panoramic views of the surgical field, albeit in 2 dimensions. Flexible endoscopes allow the device to be angled at different directions, providing better visualisation of large tumours, which may be invading superiorly into the suprasellar cistern, as well as laterally into the carvernous sinuses, hence making those spaces more accessible and potentially allowing for greater tumour volume removal [339]. However, no significant difference in the disease remission rates and peri-operative complications has been found between endoscopic and microscopic TSS [338], highlighting that TSS outcomes are significantly influenced by the skills and expertise of the neurosurgeon.

Compared with medical therapy, when surgery is used as first-line treatement in treatment-naïve patients with acromegaly results in higher remission rates [340]. Other factors that affect biochemical remission rates post-operatively include size and invasiveness of the tumour, as well as pre-operative GH levels [338]. Remission rates for microadenomas

(tumour size <10mm) approach 90% in specialist centres; however they are considerably lower in macroadenomas, particularly for tumors >20 mm in size and when there is invasion of the cavernous sinus. In these latter cases remission rates, even in specialist centres, do not exceed 50% [338, 341]. Most GH-secreting adenomas are macroadenomas and therefore overall post-operative remission rates are in the region of 42-65% [342].

In some patients, TSS is performed in order to debulk the tumour, without the prospect of obtaining remission. In these cases the aim of surgery is to relieve pressure of the tumour on the optic chiasm and/or to reduce the ambient GH levels to improve efficacy of future non-surgical treatment. Invasion of the cavernous sinus indicates that complete tumour resection is impossible and therefore post-operative remission is unlikely [343]. In these cases of persistent disease activity following initial TSS, aleternative therapeutic interventions are considered in the form of medical therapy with or without radiotherapy. Repeat surgery can be attempted in some cases, when the residual tumour is localised in the intrasellar region and is therefore accessible for resection.

Complications of TSS include new onset hypopituitarism in up to 30% of patients due to peri-operative damage to the normal pituitary tissue adjacent to the adenoma, which may require long-term replacement therapy of the affected hormone axes [344]; diabetes insipidus; bleeding; cerebrospinal fluid leak; and meningitis. Life-threatening or debilitating complications such as carotid artery dissection and visual loss are rare [345]. Lower rates of postoperative complications are seen with experienced pituitary surgeons [345]. Recurrence rates of acromegaly following initial TSS range between 2-8% over a 5-year period postoperatively [341, 343]. Recurrence indicates incomplete resection, presence of pituitary tumour in inaccessible space (i.e. cavernous sinus) or presence of adenoma tissue in the dural sellar lining, which is difficult to visualize and remove surgically.

Apart from the higher remission rates compared with other treatment modalities, successful pituitary surgery has also the additional advantages of rapid reduction in GH, as well as providing tissue samples from the tumour for histopathological analysis and further characterisation of the tumour. Histopathology is able to provide important information of

prognostic value on aggressiveness of the tumour (i.e. Ki-67 index, mitotic index), tumour invasiveness (i.e. dural invasion), degree of granulation (densely versus sparcely granulated adenomas), and presence of atypical tumour cells [346, 347].

1.7.3. Radiotherapy

Conventional fractionated conformal radiotherapy (XRT) delivers charged particles (photons) with high precision to the tumour generally using a linear accelerator. Techniques have significantly evolved as a consequence of improvements in focusing (multileaf collimator), number of beams, immobilisation, imaging, and planning. Use in the management of acromegaly has shown XRT to be highly effective in preventing growth of somatotroph tumours in 80-90% of individuals with acromegaly at 10 years [342]. In contrast, control of GH and IGF-I levels occurs at a more sedate rate with 50-60% of individuals achieving remission at 10 years [342]. In the largest individual study to date, retrospective data from 656 patients with acromegaly showed GH values <2.5 μg/l and IGF-I normalisation to be achieved in 36% and 50% of patients at 5 years respectively; and 60% and 63% at 10 years respectively [348]. Following XRT, mean GH levels decrease by around 50% every two years, however IGF-I levels decrease at a slower rate [349, 350]. The rate at which target GH levels are achieved is therefore dependent upon the ambient GH level at the time of XRT, which indicates that a significant proportion of patients will continue to have elevated GH levels for the initial years after XRT, often requiring additional medical treatment to improve the biochemical disease status.

Pituitary radiotherapy has been reported to be associated with increased risk for cerebrovascular disease, optic neuritis, visual loss and necrosis of the normal brain tissue in only occasional series and at a very low prevalence; secondary tumours (meningioma and glioma) are reported in 2-3% at 10-20 years; and a variable degree of hypopituitarism in 50-60% with long-term follow-up [180, 342, 348-351]. An excess mortality has additionally been described in patients with acromegaly who have received XRT [352]. It remains unclear whether all the described excess mortality relates to XRT or the selection of patients with

more aggressive tumours to undergo radiotherapy. With evolution of radiotherapy techniques, it is likely that many of these adverse effects will occur less frequently. Furthermore, use of advanced forms of 3D conformal radiotherapy such as intensity-modulated radiation therapy (IMRT) achieve a higher degree of target conformity and greater sparing of the surrounding tissues to radiation, and may further reduce the putative adverse effects of XRT.

Stereotactic radiosurgery (SRS) has been introduced with the aim of reducing exposure of the normal tissue surrounding the pituitary adenoma, whilst maintaining effectiveness. SRS is most frequently delivered from multiple ^{60}Cobalt gamma-emitting sources or a modified linear accelerator as a single fraction, adding patient convenience to this technique. Control of tumour growth, GH and IGF-I levels, as well as adverse sequelae of SRS do not appear to be markedly divergent from XRT [342]. Differences in outcomes of studies of SRS and XRT are potentially explicable by patient selection and pre-treatment GH and IGF-I values. SRS is used in smaller tumors, typically microadenomas of <3mm in diameter, at least 3mm from critical structures such as the optic chiasm. Studies show a similar rate of fall of GH and IGF-I levels with both techniques [353, 354]. Lower rates of radiation-induced hypopituitarism have been reported after SRS compared with conventional radiotherapy. In a large series of 136 patients with acromegaly who received SRS, new onset hypopituitarism was observed in 31% over a 5-year median period of follow up [355]. Particle radiation with proton therapy has been utilised in patients with acromegaly to further improve conformality of dose and reduce radiation exposure of the surrounding tissues [356, 357]. Larger studies and longer duration of follow up will however be required to determine if proton therapy is superior to use of photons in the control of somatotroph tumour growth, hormonal secretion, and adverse effects.

Overall radiotherapy is highly effective in tumour size reduction for most GH-secreting pituitary adenomas and lowering of GH levels. However, the latency with which normalization of GH is achieved and the high frequency of complications, particularly of radiation-induced hypopituitarism, make radiotherapy a less favourable option for use as first line treatment in acromegaly. Radiotherapy is often reserved for patients who continue to

have persistently active acromegaly following pituitary surgery, in whom a second surgical interevention is unlikely to lead to a curative result. Rarely, radiotherapy is used as first line treatment in patients who decline surgery or in whom surgery is contraindicated (i.e. high risk of general anaesthesia).

1.7.4. Medical treatment

Medical therapy is used as adjuvant therapy in acromegaly when optimal biochemical control is not achieved following pituitary surgery, while awaiting the effects of radiotherapy to be realised or as primary therapy when surgical cure is deemed unlikely (i.e cavernous sinus invasion), surgery is contraindicated or declined by the patient. Options for medical therapy include somatostatin analogues, dopaminergic analogues and growth hormone receptor antagonists.

1.7.4.i. Somatostatin analogues (or somatostatin receptor ligands)

Recognition of the importance of the physiological role of somatostatin to the negative regulation of the GH axis led investigators to study its effects on GH-secreting pituitary tumours. The action of somatostatin is mediated by 5 subtypes of somatostatin receptors (SSTR1-5), which are expressed in various cells and tissues [358]. Somatostatin inhibits a number of endocrine and exocrine functions, including inhibition of gut hormone secretion (insulin, glucagon, gastrin, vasoactive intestinal peptide, cholecystokinin); inhibition of secretion of gastric acid, pepsin, pancreatic enzymes, bile and intestinal fluids; inhibition of gastric emptying and reduction in gallbladder contractility [359]. At hypothalamic level, somatostatin blocks the release of corticotropin releasing hormone (CRH), thyrotropin releasing hormone (TRH), dopamine and norepinephrine [360].

Inhibition of GH by somatostatin is multifactorial and occurs at both a central and peripheral level. Somatostatin attenuates secretion of growth hormone releasing hormone (GHRH) by the hypothalamus, which then reduces GH synthesis and secretion by the anterior pituitary [361]. Additionally, somatotroph cells preferentially express SSTR2 and SSTR5 and therefore

somatostatin can exert a direct negative effect on the GH secretion and peripheral IGF-1 synthesis [358]. Finally, somatostatin can also act on the liver and reduce the binding of the GH to the GH receptor and therefore attenuate IGF-1 synthesis by the hepatocytes [362]. GH-secreting adenomas also express SSTR2 and SSTR5 in abundance, making somatostatin receptors a therapeutic target for reversing GH excess in acromegaly [363, 364].

Somatostatin has a short half-life in vivo of 2-3 minutes, which makes its clinical use as a therapeutic agent not viable. However, this led to the development of specific and selective long-acting somtostatin analogues (SSAs). There are currently three SSAs licenced for use in acromegaly; octreotide, lanreotide and pasireotide. Octreotide and lanreotide are considered as first generation SSAs, whereas pasireotide is a second generation SSA. Differences among the three drugs relate to selectivity of SSTR1-5-subtype binding and pharmacokinetic properties. Octreotide and lanreotide bind primarily to SSTR2 and SSTR5, whereas pasireotide shows high affinity for SSTR1, 2, 3 and 5 [365]. Pasireotide has slightly lower affinity for SSTR2 compared with lanreotide and octreotide, but 40-fold greater affinity for SSTR5 [225]. Regulation of GH secretion at the pituitary occurs primarily through SSTR2 [92, 93, 366, 367, 368] and to a lesser extent through SSTR5 [93, 366]; however SSTR1 has also been implicated [368]. Additional studies have shown that targeting both SSTR2 and SSTR5 simultaneously may produce greater results in lowering GH than SSTR2 or SSTR5 inhibition alone [92, 366].

1.7.4.i.1. First generation SSAs

Considerable long-term clinical experience has been obtained with the long-acting formulations of lanreotide and octreotide; Lanreotide Autogel (ATG) and Octreotide LAR respectively. Both preparations are usually administered on a monthly basis. Octreotide LAR is administered via intramuscular injections and the doses commonly used in clinical practice are 10, 20 and 30 mg with each injection. Lanreotide ATG is administered via deep subcutaneous injections of 60, 90 or 120mg [369, 370]. Short-acting subcutaneous octreotide is also available, however it is less efficacious compared with the long-acting preparation

[369] and less convenient from a patient's perspective, as it requires multiple daily injections to maintain its therapeutic effect on lowering GH levels. However, short-acting octreotide has been used in the break-through management of headaches associated with acromegaly [371, 372].

Clinically, Lanreotide ATG and Octreotide LAR have been shown to have similar efficacy [370]. Effects on tumour growth are remarkable, with <2% of adenoma showing significant growth on treatment [373]. The results of a meta-analysis showed tumour shrinkage in 66% of patients treated with Octreotide LAR, with a mean reduction of 50.6% in the tumour size [369]. Strict control of GH and IGF-I secretion is, however, less frequently delivered, with around 30-40% of patients achieving both a GH <2.5µg/l and a normal age-related IGF-I level [370]. Higher baseline GH and IGF-I levels are associated with a lower proportion of patients achieving target values [374]. The proportion of patients achieving control of tumour growth, symptoms, and GH/IGF-I levels is not dissimilar whether SSAs are used as primary therapy or following surgery [373]. In the PRIMARYs study 90 patients with acromegaly resulting from a macroadenoma were treated with high dose Lanreotide ATG 120mg every four weeks for 48 weeks [375]. Tumour shrinkage of >20% was achieved in 63%, and although 10% showed some degree of tumour enlargement, only 2% showed enlargement of >20%. The combined end-point of GH <2.5µg/l and normal age-related IGF-I was achieved in only 34% of patients [375].

Improvement to symptoms of acromegaly, including headaches, hyperhidrosis, soft tissue swelling, fatigue, paraesthesiae and numbness due to nerve entrapment in patients treated with SSAs, is reported in up to 80% of cases [20]. Regarding the analgesic effect of SSAs on acromegaly-related headache, it has been hypothesized that this is not purely associated with the reduction in GH levels, but is also due to inhibition of an unknown pronocuceptive peptide produced by the GH-secreting tumour which acts in a paracrine fashion [376]. Treatment with SSAs may also have beneficial effects on other complications of acromegaly, including reducing left ventricular mass, improving heart rate, cardiac function and left ventricular ejection fraction [158]. Additionally, reduction in the prevalence of obstructive

sleep apnoea has been demonstrated following treatment with SSAs [187-190], as well as improvement in the rates of dyslipidaemia [202]. No significant impact on systolic and diastolic blood pressure [158] and glucose metabolism [227] has been found with first generation SSAs. In contrast, established cardiac valve disease [152] and arthropathy [107] are not reversed by treatment with SSAs.

Response to SSA treatment depends on the degree of the expression of SSTRs, particularly SSTR2 by the GH-secreting tumour. However, testing for the presence and the subtypes of SSTRs on histopathological samples is not done routinely in clinical practice. Factors, which have been associated with positive response to SSAs, include presence of densely granulated somatotroph adenomas, older age, smaller size tumours and lower pre-treatment GH and IGF-1 values [377]. Additionally, hypointense adenomas on T2-weighted MRI, which tend to be more densely granulated tumours, are also associated with better clinical response to SSAs [378]. Surgical debulking of macroadenomas, which are not amenable to complete resection, has been shown to improve response rates to SSAs [379].

Common side effects of treatment with SSAs include asymptomatic gallstones and transient gastrointestinal (GI) disturbances (abdominal cramps, flatulence and diarrhoea) in up to 30% of patients using these medications. GI side effects tend to fade away with prolonged SSA treatment. Approximately 4% of the patients with gallstone disease related to SSA develop biochemical evidence of cholestasis [380]. Abdominal ultrasound for monitoring of gallstone disease is not routinely recommended by the American Endocrine Society, unless the patient develops symptomatic gallbladder obstruction [311]. Other side effects of SSA treatment include local skin irritation and pain at the injection site.

1.7.4.i.2. Second generation SSAs

Pasireotide is the only second generation SSA, currently approved for use in acromegaly. As a consequence of the multiligand binding (pasireotide binds too SSTR1-3 and SSTR5), pasireotide has been considered a promising candidate to improve control of GH and IGF-I levels in patients with acromegaly. Pasireotide acts to inhibit GH secretion at the pituitary via

interaction with multiple SSTRs, but additionally may have peripheral effects to reduce GH-induced IGF-I production by the liver [362].

The initial phase II clinical study comparing subcutaneous pasireotide and octreotide suggested pasireotide to have the greater efficacy in controlling GH and IGF-I levels [381]. The follow-up phase III study of the long-acting formulation, pasireotide LAR, showed greater efficacy than octreotide LAR in achieving target GH levels of <2.5μg/l and normal IGF-I levels (31.3 Vs. 19.2% respectively) in patients with uncontrolled acromegaly who had not previously received medical therapy [231]. Additionally, pasireotide LAR has been shown to induce biochemical remission in up to 20% of patients who remain uncontrolled during long-term therapy with first generation SSAs [232].

Adverse events were similar for pasireotide and first generation SSA with the exception of hyperglycaemia, which was significantly more frequent with pasireotide, occurring in up to 67% of patients [231, 232]. The development of diabetes and hyperglycaemia with pasireotide is an obvious concern, as both type 1 and type 2 diabetes themselves are associated with an increased standardised mortality ratio (SMR). However, it does appear that the diabetes associated with pasireotide may be reversible with treatment discontinuation [382], and that mechanistically it differs from other forms of diabetes.

1.7.4.ii. Dopaminergic agonists

Dopaminergic agonists have primarily been used in the management of hyperprolactinaemia and to inhibit lactation [383]. Paradoxically, it was observed that dopaminergic drugs also show suppression of GH secretion in patients with somatotroph tumours [384] and as a consequence these agents have been trialled in the management of acromegaly, both as monotherapy and also in combination with SSA therapy. Dopamine receptors have been identified not only in mixed GH and prolactin secreting adenomas, but also in GH-secreting tumours [385, 386]. Additionally, it has been shown that dopamine subtype 2 (D2) receptors can interact with SSTR5 to form hetero-dimers with enhanced functional activity [387].

The first dopaminergic drug to be used in the management of acromegaly was bromocriptine. Studies in small numbers of patients showed very limited efficacy with only around 10% achieving biochemical target [388]. With the advent of cabergoline and quinagolide for treatment of prolactin-secreting tumours came increased efficacy, fewer side effects, and a more acceptable dosing regimen. Early studies of the use of cabergoline in patients with acromegaly included small numbers of patients and showed variable efficacy and biochemical disease control [389-393]. Inadequate studies, combined with the historically poor efficacy of bromocriptine in individuals with acromegaly has led to this class of drugs being considered only infrequently as monotherapy for controlling GH and IGF-I levels, with their use now generally being accepted for patients with only mild disease activity, or as an addition to SSA therapy [311].

A meta-analysis of 10 studies (n=160 patients) in 2011 reviewed the published data regarding the effects of cabergoline monotherapy in the management of acromegaly. All but one of the studies were open, and none were randomised or placebo controlled. Cabergoline was used first line in 21% of patients, and the overall mean dose was 2.6mg / week. Baseline GH and IGF-I levels were 16 ± 34 mcg/L and IGF-I 82.9 ± 36.5 nmol/l respectively. Target GH levels of <2.5 mcg/l and a normal age-adjusted IGF-I were achieved in 48% and 34% respectively [394]. Patients who achieved target GH and IGF-I levels had lower baseline IGF-I levels and elevated prolactin levels at baseline. In the same meta-analysis, addition of cabergoline to the management of patients with uncontrolled disease whilst on treatment with SSAs, led to normalisation in IGF-1 in 52% of the patients who were on the combined therapy. Mean GH levels significantly reduced from 7.4 mcg/L to 3.6 mcg/L; however the exact of proportion of patients who achieved the target GH of <2.5 mcg/L on the combined treatment was not reported [394]. Data from the UK Acromegaly Register examined 355 courses of treatment with cabergoline, and of these 36% achieved a target GH <2.0μg/l and a normal IGF-I level [374].

The effect of cabergoline on tumour size has also been assessed in some studies. Tumour shrinkage has been reported in both patients with mixed GH/prolactin secreting adenomas and

also in patients with pure GH-secreting tumours [389-392]. However, reduction in tumour volume has been associated with higher baseline prolactin and IGF-1 levels [394].

Cabergoline has been well-tolerated overall. Common, mild side effects that have been reported include GI upset (nausea, constipation), headaches, orthostatic hypotension, dizziness, mood disorders and nasal congestion [394]. Cardiac valve function was not assessed in the studies for the use of cabergoline in patients with acromegaly. Concerns about the cardiac safety of dopaminergic agnoinists have been previously raised and stem from reports on cardiac valvulopathy in patients with neurological disorders (i.e. Parkinson's disease) treated with high doses of cabergoline; however no increased risk for cardiac valve dysfunction has been observed with lower doses of cabergoline commonly used in the management of patients with prolactinomas [395]. Additionally, patients should be aware about the risk of impulse control disorders associated with dopaminergic agonists, although these have been more frequently reported in patients with Parkinson's disease and with higher doses of dopamine agonists [396].

In the 2014 clinical practice guidelines, the American Endocrine Society recommended the use of cabergoline in patients with modest elevation of GH and IGF-1 levels, with or without hyperprolactinaemia [311]; however considering that dopamine agonists provide similar control rates of GH and IGF-1 to those observed with SSAs, it may therefore be appropriate that these drugs are trialled earlier in the management of acromegaly based on efficacy and cost-effectiveness. Current NHS costs for 28 days therapy with Octreotide LAR or Lanreotide ATG approximate to £500-£1000. In contrast cabergoline at a weekly dose of 2-3mg equates to £25-£40 for the same period. In any case, cabergoline can be used in conjunction with SSAs, in cases of acromegaly where SSA monotherapy has failed to achieve biochemal remission.

1.7.4.iii. Growth Hormone Receptor Antagonist (Pegvisomant)

Pegvisomant is a genetically modified analogue of human growth hormone, which acts as a highly selective growth hormone receptor antagonist, blocking the GH receptor signalling and

subsequent IGF-1 production, thus eliminating the actions of GH to the peripheral tissues [397]. As pegvisomant only acts at the GH receptor level in the periphery, it has no direct central effects on the pituitary gland and the GH-secreting adenoma. Therefore, GH excess persists and IGF-1 is the sole biochemical marker to assess drug efficacy. In fact, the loss of negative feedback by the lowering of IGF-1 leads to an increase in GH levels [398].

The two seminal studies of pegvisomant monotherapy demonstrated efficacy, defined by normalisation of age-related IGF-I levels, of 89-97% of patients [399, 400]. In contrast, real world data from the ACROSTUDY showed lower rates of IGF-I normalisation, which occurred in 63.2% of patients treated with pegvisomant for 5 years, however, this likely reflects inadequate titration of pegvisomant dosage and lower rates of compliance than seen in the controlled clinical studies [401]. The first study in 2005 examining the addition of pegvisomant to SSA therapy showed control of IGF-I in over 95% of individuals [402]. Similar studies have confirmed these excellent outcomes with almost all patients showing normalisation of IGF-I levels [403]. Superior outcomes have also been reported by the combination of pegvisomant with cabergoline than using each of these agents as monotherapy. In a small prospective of 24 patients with acromegaly, cabergoline as monotherapy at a dose of 3.5mg / week, achieved normal IGF-1 in only 11% of cases. The addition of pegvisomant increased the prevalence of IGF-1 control to 68%; however discontinuation of cabergoline led to a decrease in the normal IGF-1 rates to 26%, indicating that pegvisomant and cabergoline can act synergistically to improve treatment outcomes [404].

Increase in the adenoma size has been reported in 5-7% of patients received treatment with pegvisomant [405]. However, it remains unclear whether this is a drug-related effect due to the loss of the negative feedback to the pituitary by the decrease in IGF-1, or whether this represents the disease natural history, as in clinical practice pegvisomant has mainly been used in cases of more aggressive GH-secreting pituitary adenomas which are resistant to “conventional” treatment with SSAs. However, with combinations therapies of SSAs with pegvisomant, concerns over enlargement of the pituitary adenoma residuum have not been

realised [403]. The American Endocrine Society recommends close pituitary imaging surveillance for patients treated with pegvisomant, which involves imaging at 6 and 12 months after the initiation of treatment and annually thereafter, providing that no change in the size of the residual adenoma was seen in the first year of treatment [311]. Additionally, they also recommend against using pegvisomant in patients with large pituitary tumours abutting the optic chiasm [311].

Pegvisomant is administered via subcutaneous daily injections of 10, 15 or 20mg to a maximum dose of 30 mg per day. Side effects of treatment include injection site reactions in approximately 2% of patients (local discomfort, reversible lipohypertrophy or lipoatrophy) [406]; and abnormal rise in liver enzymes in 9% of patients [407]. Monitoring of liver function is therefore recommended and in case of a greater than 3-fold rise in serum transaminases, discontinuation of pegvisomant is advised [311].

1.7.4.iv. Oestrogen and Selective Estrogen Receptor Modulators

Reduction in IGF-1 levels has been observed with oral oestrogens or selective estrogen receptor modulators (SERMs), which have been used alone or in combination with SSAs in a limited number of studies [408, 409]. An increase in GH has been reported, suggesting that these medications do not act at the pituitary or hypothalamic level [410]. It has been proposed that oral oestrogens lower the GH-induced synthesis of IGF-1 in the liver [411]. A meta-analysis for the use of oestrogen and SERMs in 63 patients with acromegaly from 6 different observational studies, showed that a significant reduction in IGF-1 was achieved [412]. The decline was greater in women receiving oral oestrogen, followed by women on SERMs. The reduction in IGF-1 in men treated with SERMs was not statistically significant, possibly due to the small sample size [412]. Therefore, it appears that SERMs have a less potent effect on lowering IGF-1 compared with oral oestrogens. This is likely due to the weaker oestrogenic action of SERMs, as their function via the oestrogen receptor is tissue-specific, acting either as receptor agonist or antagonist at different target tissues [411]. Overall the evidence suggests that oestogen and SERMs may have a role as a low cost, adjuvant therapy in patients

with active acromegaly despite other therapies and particularly in women with persistently elevated IGF-1 levels. However due to the limited available data, the use of these drugs in management of acromegaly is not advocated by the current practice guidelines.

1.7.4.v. New therapeutic agents

Novel agents for the management of acromegaly have been under development in the recent years. These include new forms of somatostatin analogues, which target GH hypersecretion and other agents targeting the GH receptor. The oral octreotide capsule has been synthesized with a transient permeability enhancer, which facilitates intestinal absorption of octreotide [413]. A phase III trial showed that oral octreotide is efficaceous, achieving biochemical therapeutic targets (GH <2.5 mcg/L and IGF-1 < 1.3 times the upper limit of normal) in 62% of patients over the 13-month study period. Additionally, the safety profile was similar to the injectable SSAs [414]. Similar to the injectable octreotide, oral octreoride has affinity to SSTR2 and SSTR5, but has the benefit of oral administration, instead of intramuscular injection, which for some patients may be a more attractive therapeutic option. In the phase III study oral octreotide capsule was administered twice daily [414].

CAM2029 (octreotide sc depot) contains a hydrophilic octreotide peptide and was designed to address issues related to the pharmacokinetics of octreotide LAR and also improve patient's experience with injectable treatment. A phase I trial showed drug bioavailability was greater with CAM2029 compared octreotide LAR and led to a greater reduction in IGF-1 levels [415]. Additional benefits of CAM2029 include administration via a thinner needle; the preparation is available in prefilled syringes and, unlike octreotide LAR, it does not require reconstitution prior to its administration or storage in a refridgerator [415]. The frequency at which CAM2029 needs to be administered has not been determined yet, but it is likely to be every 4 weeks, similar to octreotide LAR [416]. A phase II trial on 12 patients with either acromegaly or neuroendocrine tumours was completed in 2016 and showed that higher plasma octreotide levels were achieved with CAM2029 rather than octreotide LAR, biochemical disease control was maintained in patients with acromegaly, while patients with

functioning neuroendocrine tumours continued to have symptoms control with CAM2029 [417].

DG3173 (or somatoprim) is a selective somatostatin analogue, with high affinity for SSTR2, SSTR4 and SSTR5, which was found to lower GH levels, without inhibiting insulin secretion [418]. Therefore it has been speculated that it carries a lesser potential of causing complications of glucose metabolism (hyperglycaemia or diabetes) compared with other SSAs and particularly pasireotide. An in vitro study on GH-secreting adenoma cultures derived from patients with acromegaly showed that DG3173 suppressed GH secretion in a larger number of tumours than octreotide (10/21 versus 5/21 respectively) [419]. Additionally, it was noted that sparcely granulated tumours were more likely to respond to DG3137 [419], which is in contrast with the first generation SSAs, where better response rates are observed in patients with densely granulated GH-secreting adenomas. A phase II clinical trial assessing the effect of of subcutaneous infusions of 3 doses of DG3173 on 8 patients with untreated acromegaly was completed in 2016; however the results were not published in a peer-reviewed journal, possibly due to the fact that the initial recruitment target of 20 patients was not achieved [420].

A subcutaneous octreotide hydrogel implant, providing continuous release of octreotide for 6 months has also been developed as an attempt to reduce the variation in the plasma octreotide levels as seen with the conventional injectable therapy with the 1st generation SSAs and also to increase patients' convenience by avoiding monthly intramuscular injections [421]. The efficacy and safety of the octreotide implant was assessed in a phase III open label trial, in which 163 patients with acromegaly who were already on treatment with octreotide LAR were randomized to either the 6-monthly octreotide implant or to continue with the monthly octreotide LAR injections. At the end of the 6-month study period similar rates of GH suppression were observed between the two study arms. Higher rates of diarrhoea and headaches, but lower rates of cholecystitis were reported from patients on the implant compared with those on octreotide LAR [421]. The octreotide implant is not currently commercially available.

In contrast to the previous agents, which target the somatostatin receptors in order to inhibit GH secretion, an antisense oligonucleotide, ATL1103, is currently under development. ATL1103 has high affinity for the GH receptor mRNA inhibiting its translation [422]. A phase I study in healthy volunteers showed that administration of ATL1103 resulted in reduction in IGF-1 and GH-binding protein [423]. A phase II clinical trial on 26 patients with acromegaly was recently completed. A significant decline by a median of 27.8% in the IGF-1 values was observed after 13 weeks of treatment and the reduction was more pronounced in the groups of patients who were randomized to the twice-weekly administration regime compared with the once-weekly group. Similar to pegvisomant, a rise in serum GH was observed due to the lack of negative feedback to the hypothalamic-pituitary level. Although the drug was well-tolerated, almost 85% of patients had mild to moderate injection site reactions [424]. Current data on ATL1103 serve as proof of concept and further placebo-controlled studies using higher doses of ATL1103 and for longer duration are in progress.

1.8. Quality of Life in patients with Acromegaly

Despite the advancements in the treatment of acromegaly, there is a plethora of studies reporting that patients with acromegaly have impaired quality of life (QoL) compared with the general population, irrespective of the disease clinical status. Although some improvement to the QoL following treatment of acromegaly has been reported, QoL remains reduced in both patients with active disease and disease remission compared with the normal population [425, 426].

Health-related quality of life (HR-QoL) refers to the impact of an illness and its treatment on the quality of life, as this is perceived by the patient. It is a complex and multi-dimensional concept, which encompasses the domains of physical, mental and emotional well-being and social functioning [427]. HR-QoL is highly dependent on the patient's expectations, personal goals and desires, as well as the cultural background and social environment. Improving QoL is one of three target areas recognized by the World Heath Organization (WHO), in the

management of patients with chronic diseases, alongside reducing mortality and morbidity [428].

A variety of disease-specific and generic questionnaires have been implemented to assess HR-QoL in patients with acromegaly. The Acromegaly Quality of Life (AcroQoL) questionnaire is a 22-item questionnaire, which was developed specifically for patients with acromegaly. It is divided into the physical domain comprising of 8 questions and the psychological domain comprising of 14 questions. The psychological dimension encompasses the appearance (7 questions) and personal relationships (7 questions) domains [429-431]. Generic questionnaires which have been frequently used to assess QoL in patients with acromegaly include the Psychological General Well Being Scale (PGWBS), the EuroQoL (including EQ-5 Dimesions and the EQ-Visual Analogue Scale), the Short Form 36 (SF-36) and the Nottingham Health Profile (NHP). Each of the generic QoL questionnaires contains a variety of questions designed to assess different aspects of physical and emotional well-being [432-436].

There is significant heterogeneity in the methodology of the studies evaluating quality of life in patients with acromegaly. The majority are cross-sectional studies assessing QoL in mixed patient populations with active acromegaly and disease remission. Additionally, there are a number of interventional studies, which have assessed changes in QoL in patients with active disease or treatment-naïve patients before and after surgical and/or medical therapeutic interventions. Finally, some observational studies have evaluated QoL longitudinally, at multiple time-points during the disease course. Therefore, interpreting the results of these studies in a unifying way becomes challenging, as different factors may influence QoL at different stages of acromegaly.

1.8.1. Impaired QoL due to physical symptoms

Patients with both active acromegaly and disease remission have reduced physical function compared with the general population [107, 112, 437-439]. Arthropathy and joint pain are the main factors which influence negatively the domains of physical activity and function, as

these assessed by the various disease-specific and generic questionnaires. Joint pain is reported by the majority of patients with acromegaly, despite disease control [437] and studies have shown that patients who experience arthralgia have significantly lower QoL outcomes compared with patients without joint pain [107, 112]. Even when compared with patients with other types of pituitary tumours (i.e. non-functioning pituitary adenomas or prolactinomas), patients with acromegaly were found to experience higher levels of physical pain, leading to worse QoL outcomes, with lower scores in the domains of physical function, vitality, social functioning and general health perception [437, 440, 441]. In addition to joint pain, headache and neuropathic pain due to soft tissue swelling and nerve entrapment syndromes (i.e. carpal tunnel syndrome) have also been shown to negatively influence QoL [438, 442, 443].

1.8.2. Impaired QoL due to psychological symptoms

Higher rates of affective disorders (anxiety and depression) have been found in patients with acromegaly compared with controls with and without other chronic somatic disorders selected from a sample representative of the general adult population in Germany (odds ratio 2.0 and 4.4 respectively) [444]. Anxiety and depression have been shown to be superior predictive factors of impaired QoL compared with biochemical markers of disease control and it has been recommended that appropriate interventions to address psychopathology in patients with acromegaly may be one pathway to improve QoL in these individuals [445]. Delay in the diagnosis of acromegaly (defined as the time between the patient's first medical visit due to acromegaly-related symptoms and establishing the diagnosis of acromegaly) was found to be correlated with poorer psychological QoL outcomes, such as depression, sleep disorders and worse body image perception [446]. Negative illness perceptions, which reflect the way patients view their disease and its influence on their lives, have been identified in patients with acromegaly and are strongly associated with impaired QoL [447, 448].

The domain of physical appearance is one of the most affected areas in the QoL assessment, leading to psychological distress and reduced QoL [449, 450]. This is probably expected

considering the extent of the somato-skeletal alterations associated with acromegaly, which are irreversible to a significant degree. Almost 50% of patients with long-term disease remission reported self-consciousness regarding their appearance, with facial characteristics being the main cause of self-consciousness [451].

1.8.3. Impaired QoL due to neurocognitive symptoms

Impaired cognitive function in the context of short and long-term memory deficits has been demonstrated in treatment-naïve patients with active acromegaly based on data from quantitative electroencephalogram and low-resolution brain electromagnetic tomography [452]. These changes in neurocognitive function do not reverse following successful pituitary surgery and biochemical remission [453]. Additionally, self-reported perception of cognitive function has been found to be impaired in patients with active acromegaly, particularly regarding the functions of concentration/distractibility and ability to learn [454]. Impairment in delayed memory and decision making have also been identified in patients with acromegaly and have been associated with anxiety and depression, suggesting that cognitive dysfunction may be contributing to the psychopathology of these individuals and subsequently to the reduced QoL [455].

1.8.4. The effect of acromegaly treatment on QoL

Prospective studies, assessing QoL in patients with acromegaly before and up to 12 months after trans-sphenoidal surgery have produced mixed results. Most data are in favour of an improvement to the QoL following surgery [456, 457] from as early as 3 months [456]. Another study reported improvement only in the mental component of the QoL assessment but not in the physical or the role-social components [457]. No significant improvement to QoL, as assessed by the AcroQoL questionnaire before and 12 months after pituitary surgery has also been reported [458].

Several studies have shown that pituitary radiotherapy is associated with adverse HRQoL outcomes [439, 440, 459, 460]. The negative impact of radiotherapy on QoL is likely

multifactorial. One potential mechanism is via the radiotherapy-induced hypopituitarism. An inverted U-shaped association between HRQoL and nadir GH levels on the OGTT post-treatment of acromegaly has been described [459]. The best HRQoL outcomes were found in patients with nadir GH between 0.3 and 1.0 mcg/L post OGTT. In contrast patients with nadir GH levels below 0.3 mcg/L (suggestive of acromegaly overtreatment and possible GH deficiency) or above 1.0 mcg/L (suggestive of on-going active disease) had less favourable QoL scores [460]. Patients who develop GH deficiency following treatment for acromegaly have been found to have impaired QoL, which improves with GH replacement therapy [461, 462]. In addition to hypopituitarism, pituitary radiotherapy may be associated with reduction in neurocognitive functioning, similar to cancer survivors who have received cranial irradiation for brain tumours [463].

Prospective studies comparing QoL in patients with active acromegaly (mostly treatment naïve patients) at baseline and up to 12 months of treatment with long-acting somatostatin analogues, showed improvement to QoL with both Lanreotide Autogel and Octreotide LAR [464-467]. In the PRIMARYS study, in which 90 treatment-naïve patients with acromegaly were included, a greater improvement to HR-QoL was noted amongst patients who achieved biochemical remission with Lanreotide Autogel monotherapy, compared with patients who still had biochemically active disease after 1 year of receiving Lanreotide Autogel as primary treatment [464]. Switching treatment from Octreotide LAR to Lanreotide Autogel did not alter QoL scores, suggesting there is no superiority between the two long-acting somatostatin analogues with regards to HR-QoL outcomes [468]. Similarly, when patients with acromegaly and suboptimal biochemical control were switched from Octreotide LAR to Pasireotide, no change in QoL was found [382]. Addition of pegvisomant to somatostatin analogue therapy in both patients with suboptimal acromegaly control [469] and those with normal IGF-1 [470] values led to a significant improvement to QoL. Furthermore, patients with inadequate biochemical disease control also showed improved QoL outcomes following 40 weeks of pegvisomant monotherapy [469].

In contrast to the above, a number of cross-sectional studies performed in patients who have previously received multi-modality treatment for acromegaly, showed worse QoL scores amongst patients who required long-term treatment with somatostatin analogues in order to achieve biochemical remission [471-473]. This was shown to be related to negative medications beliefs leading to more negative illness perceptions, with patients on long-term SSA attributing more symptoms to acromegaly and therefore perceiving worse QoL and a more chronic course of the disease [472]. Additionally, a large proportion of patients on long-term SSA treatment report injection-related symptoms and injection-site reactions, with 70% of patients describing pain lasting up to one week after the injection and over 30% feeling loss of independence as a result of the regular injections [473]. Therefore, medical therapy when introduced for the management of patients with active acromegaly has a positive impact of QoL in the short-term, as indicated by the previously mentioned interventional studies, possibly by improving symptoms associated with disease activity; however when used long-term aiming for biochemical remission, is associated with worse QoL outcomes due to more negative illness perceptions and a significant burden due to injection-related adverse effects and symptoms

1.9. Mortality in patients with Acromegaly

Acromegaly has been associated with increased overall mortality compared with the general population. Two meta-analyses published in 2008 showed a mean standardised mortality ratio (SMR) of 1.72 (95% confidence interval 1.62-1.83) [474] and 1.70 (95% CI 1.5-2.0) [475] respectively. However, it has been well-recognised that overall mortality rates in acromegaly have been reducing with time, reflecting the advancements in the therapeutic interventions and the higher remission rates with modern treatments. Prior to the introduction of trans-sphenoidal surgery in the 1970s, radiotherapy was the first line treatment for patients with acromegaly. Cure rates of the disease improved further with the more widespread use of somatostatin analogues in the 1990s. The reduction in mortality rates is reflected in a more recent meta-analysis from 2018, in which the SMR from clinical studies published after 2008

is not significantly higher compared with the general population (SMR 1.35, 95% CI 0.99-1.85), however meta-analysis of studies published before 2008 demonstrated an increased SMR (SMR 1.76, 95% CI 1.52-2.04) [179].

The positive impact of modern therapy in mortality rates in patients with acromegaly is demonstrated in a study comparing mortality outcomes in a cohort of patients from Italy, where somatostatin analogues were widely used versus a cohort of patients from Bulgaria, where use of somatostatin analogues was limited (64% vs. 8%, in the SSA use rates respectively). The study showed elevated mortality rates in Bulgaria (SMR: 2.0, 95% CI 1.54-2.47), whereas in Italy mortality was similar to the background population (SMR: 0.66, 95% CI 0.27-1.36) [476].

Despite the above, there still remains some controversy in the recent studies, whether acromegaly continues to be associated with increased mortality. Data from the French Acromegaly Registry showed a SMR similar to the general population (SMR 1.05, 95% CI 0.7-1.42) based on data from 999 patients between 1999 and 2012 [150]. In contrast, data from the Swedish National Health Registries shows that overall mortality is decreasing, but continues to remain higher compared with the general population [477].

Main causes of death in patients with acromegaly include cardiovascular, cerebrovascular and respiratory diseases. High SMRs for all the above causes have been found in a meta-analysis of studies published both before and after 2008 [179]. The collective SMR has been calculated at 1.95 (95% CI 1.58-2.40); 2.76 (95% CI 1.90-4.02); and 2.48 (95% CI 1.80-3.41) for cardiovascular, cerebrovascular and respiratory diseases respectively [179]. The SMR due to malignancies was not elevated in the studies published before 2008 (1.33, 95% CI 0.99-1.79); however a rise to the number of deaths due to malignant diseases was noted in the studies published after 2008, with an increase to the SMR at 1.48 (95% CI 1.15-1.90); however this may be related to the improved survival rates of patients with acromegaly, whose mortality is approaching the one of the general population, rather than reflecting a true association between acromegaly and risk of malignancy [179]. This is supported by the range of different types of cancer reported as cause of death in patients with acromegaly, including

brain, lung, liver, pancreatic, breast, ovarian, prostate, haematological cancer and melanoma, which are not malignancies that have been commonly associated with acromegaly [300, 335, 352, 477, 478].

Factors adversely influencing mortality in patients with acromegaly include increasing age and previous radiotherapy [479], with the latter being associated with increased risk of cerebrovascular disease. Presence of hypopituitarism and particularly ACTH deficiency following treatment with acromegaly has also been associated with increased mortality [352]. Finally, increased mortality was observed in patients with active acromegaly, however this becomes indistinguishable to the background population in patients with disease remission [474, 475, 479, 480]. Over the years there has been a change to the biochemical criteria used to define disease remission in acromegaly. In a meta-analysis of studies published before 2008, GH levels >2.5 mcg/L were associated with increased mortality, whereas patients with GH <2.5mcg/L and IGF-1 levels within the normal range revealed SMRs similar to the general population [475]. However, with newer and more sensitive GH assays a GH threshold of <1mcgL has been shown to normalize mortality in patents with acromegaly [335, 479, 481]. This lower threshold has also been endorsed by the American Endocrine Society in the 2014 Clinical Practice Guidelines, as a therapeutic goal following treatment for acromegaly, alongside a normal age-specific IGF-1 [311]. In contrast, data on IGF-1 as a predictor of mortality have been less consistent, with some studies showing an association between IGF-1 and mortality [138, 480], however this association was not present in other studies [180, 352, 476, 478, 481].

1.10. Hypothesis and Aims of the Thesis

Despite the therapeutic advancements in the management of patients with acromegaly, there still remains significant morbidity and mortality. Of the different complications associated with acromegaly, arthropathy has the highest prevalence and is one of the main determinants of quality of life in these patients, while cardiovascular disease remains one of the leading causes of mortality in acromegaly patients. The aim of this thesis is to explore further the

unresolved issues of impaired quality of life, arthropathy and cardiovascular mortality associated with acromegaly and in particular to:

- Establish whether quality of life changes over time in patients previously treated for acromegaly and who have biochemically stable or improved disease.
- Identify positive and negative predictors of quality of life in patients with acromegaly.
- Characterise the joint alterations in patients with acromegaly, by evaluating subchonadral knee bone shape (a surrogate marker of osteoarthritis) using a novel imaging technique based on automated segmentation of MR images of knee bones and calculation of bone area using active appearance models.
- Identify factors, which may be associated with altered bone shape in patients with acromegaly.
- Assess for any correlation between knee bone shape and patient-reported outcomes and markers of quality of life.
- Explore whether acromegaly is associated with increased thrombotic potential, by evaluating the effects of GH/IGF-1 excess on clot formation and lysis, as a potential mechanism for the increased cardiovascular mortality observed in patients with acromegaly.
- Identify factors which may be associated with adverse clot structure properties in patients with acromegaly.

CHAPTER TWO

ASSESSING QUALITY OF LIFE IN PATIENTS WITH ACROMEGALY: RESULTS FROM A 5-YEAR PROSPECTIVE STUDY

2.1. ABSTRACT

Objective: Patients with acromegaly demonstrate impaired quality of life (QoL), but data on long-term changes in QoL in treated acromegaly are limited. This study evaluates and identifies factors that influence QoL in patients with long-term biochemical remission.

Design: This study consists of a cross-sectional arm to compare QoL between patients with treated and controlled acromegaly and healthy controls; and a longitudinal arm to assess QoL changes in patients with biochemically stable disease during 5.7±0.6 years of follow-up.

Patients: 58 patients and 116 matched controls (ratio 1:2) were recruited for the cross-sectional arm; 28 patients completed the longitudinal arm.

Measurements: Three generic questionnaires [Psychological General Well-Being Schedule (PGWBS), 36-item Short-Form (SF-36), EuroQoL (EQ-5D)] and the disease-specific acromegaly QoL questionnaire (AcroQoL) were applied for QoL evaluation.

Results: QoL assessment was performed 11.6±8.2 years following diagnosis and treatment of acromegaly. Patients with treated acromegaly had lower QoL scores compared with controls in all questionnaires with the exception of the PGWBS "Anxiety" subscale. The "Appearance" subscale of the AcroQoL and the "physical function" subscales of the remaining questionnaires were the most underscored domains. No difference in the total and subscale scores of all questionnaires was observed between baseline and follow-up assessment, with the exception of the SF-36 "Physical Function", where a decline was found between baseline and follow-up (58.5±24.7% vs. 43.1±31.1%; p=0.002). Duration of IGF-1/GH control was positively correlated with QoL scores in most questionnaires at baseline, whereas use of GH lowering therapy at the time of QoL assessment was a negative predictive factor of QoL.

Conclusions: Patients with biochemically controlled acromegaly demonstrate impaired quality of life, which persists despite long-term disease control, is primarily consisted of impaired physical function and secondly of impaired psycho-social well-being. Duration of biochemical disease control and current use of GH lowering therapy were the predominant factors determining patients' QoL.

2.2. INTRODUCTION

Patients with acromegaly experience considerable morbidity in terms of lethargy, sweats, arthralgia, headaches, and a reduction in general well-being. One of the most important issues is the impact of acromegaly on health-related quality of life (HR-QoL). Several cross-sectional studies have reported reduced quality of life (QoL) in patients with acromegaly [430, 450, 459, 471, 482]. Although treatment of active acromegaly has shown improvement in QoL [470, 483, 484], significantly impaired QoL has been found to persist in patients with long-term disease remission in cross-sectional studies [107, 112, 439, 448, 485], suggesting that biochemical control does not necessarily translate into normalisation of QoL as perceived by patients themselves. Even when compared with patients with other pituitary tumours, patients with acromegaly demonstrate worse QoL scores [440]. Previous radiotherapy [107, 460, 482], treatment with somatostatin analogues [471], longer duration of active disease [107], arthropathy [107, 112], biochemically active disease [483, 486, 487] and female gender [471] have been shown to negatively influence QoL in patients with acromegaly.

Despite a plethora of cross-sectional studies assessing QoL in patients with acromegaly, the number of prospective studies evaluating changes in the HR-QoL in patients with long-term biochemical disease control is limited. In the only other long-term prospective study of patients with controlled acromegaly, van der Klaauw et al. showed no change in QoL scores in most of the questionnaires subscales during a 4-year follow-up period, however a significant deterioration in the QoL scores was noted in five QoL subscales, including physical and social functioning, physical fatigue, psychological well-being and personal relationships [460]. Given the challenge in managing the long-term morbidity associated with acromegaly, a two-stage study was conducted to evaluate HR-QoL in patients with treated acromegaly and long-term biochemical disease control. In the first stage of the study (cross-sectional), patients' QoL was compared with that of a control population from the same geographic and socio-economic background. In the second stage (longitudinal), QoL parameters were assessed over a 5.7-year follow-up period, to evaluate changes in HR-QoL in patients with sustained biochemical disease control and no deterioration in GH and IGF-1

levels, and also to determine factors that influence HR-QoL outcomes in patients with treated acromegaly.

2.3. SUBJECTS AND METHODS

2.3.1. Participants' Recruitment

HR-QoL data on patients previously diagnosed and treated for acromegaly were collected prospectively. Patients were approached at the time of their routine endocrine follow-up clinic appointments at Leeds Teaching Hospitals. HR-QoL assessment was performed by the disease-specific Acromegaly Quality of Life (AcroQoL) questionnaire and three validated generic questionnaires: the Psychological General Well-Being Schedule (PGWBS), the 36-item Short-Form (SF-36) and the EuroQoL (EQ-5D). Patients were recruited during a 7-year period, between 2008 and 2015. Patients recruited before 2010 were asked to provide two sets of responses to the questionnaires within a time interval of minimum 5 years.

Data were also collected from a control population, using the three generic questionnaires (PGWBS, SF-36 and EQ-5D). Considering that the AcroQoL has been designed as a disease-specific questionnaire, it was not used for the health status assessment in healthy volunteers. The control population was derived from the hospital staff and patients' relatives, in order to ensure that both patient and control groups came from a similar socio-economic background and geographical area. To minimise selection bias, hospital staff of different professional backgrounds were included in the control group (doctors, nurses, healthcare assistants, admin/clerical staff, laboratory staff, porters, domestic staff). Individuals with a history of acute illness within the last 3 months were excluded; however, a history of a chronic, stable, non-debilitating illness was not an exclusion criterion. Examples of chronic, stable conditions include patients with a history of hypertension, dyslipidaemia, asthma, primary hypothyroidism, who have been on long-term treatment with appropriate disease control. Each patient was individually matched for age and gender with controls at a ratio 1:2

(patients: controls). Data for the control population were collected between September and December 2015.

A favourable opinion from the local Research Ethics Committee (REC) was obtained (REC reference number: 08/H1313/36).

2.3.2. Data Collection

Patients' medical records were reviewed and data relevant to patients' history of acromegaly collected, including year of diagnosis of acromegaly, treatment modalities applied (pituitary surgery, radiotherapy and/or medical therapy), duration of active disease (estimated from the time of onset of symptoms of acromegaly until biochemical disease control was achieved), duration of disease remission, GH and IGF-1 levels at the time of completion of the QoL questionnaires (to reflect the patient's current disease activity), presence of hypopituitarism as a result of the pituitary tumour or treatment thereof, other co-morbidities and concomitant medications. Remission of acromegaly was defined based on the American Endocrine Society clinical practice guidelines (GH <1mcg/L and IGF-1 within the reference age according to patient's age) [311].

2.3.3. Inclusion / Exclusion criteria

Patients with established diagnosis of acromegaly, above the age of 18 years and able to provide informed consent were included in the study. In case of hypopituitarism, only patients who had been on stable hormone replacement with sex steroid hormones, hydrocortisone, levothyroxine and desmopressin (for cranial diabetes insipidus) when appropriate, for at least three months prior to the completion of the questionnaires were included. Patients with learning/reading difficulties, dementia, terminal illness or history of acute illness within the last 3 months, prior to questionnaire completion, were excluded from the study.

2.3.4. Study Design

The study was divided into two arms: a cross-sectional arm comparing HR-QoL outcomes from patients' baseline responses to the questionnaires with those obtained from healthy controls; and a longitudinal arm, to compare patients' responses at baseline and after a five-year follow-up period, to identify changes in the HR-QoL in patients with previously treated acromegaly and no deterioration in the biochemical markers (GH and IGF-1), which are used as indicators of disease activity in the long-term. When assessing for changes in QoL scores during the follow-up period, the minimally important difference (MID) was applied to define improvement or deterioration in QoL scores between baseline and follow-up QoL evaluation. MID was defined as a difference greater than 50% of the baseline standard deviation for the corresponding baseline QoL scores [464].

2.3.5. Study Questionnaires

The questionnaires used to assess HR-QoL outcomes in our cohort included the: (i) AcroQoL, (ii) PGWBS, (iii) SF-36 and (iv) EQ-5D. Previous studies have confirmed the validity of the above questionnaire [430, 432, 433, 488-490].

2.3.5.i. AcroQoL

The AcroQoL is the only disease-specific questionnaire to measure HR-QoL outcomes in patients with acromegaly [429]. It consists of 22 items; 8 of which evaluate physical aspects and 14 which evaluate psychological aspects. The psychological scale is further subdivided in the physical appearance and personal relationships subscales. There are 5 possible answers for each item on the AcroQoL, which are scored in a 1 to 5 scale, with higher scores representing better HR-QoL outcomes [431].

2.3.5.ii. Psychological General Well-Being Schedule (PGWBS)

The PGWBS is a validated generic QoL questionnaire which contains 22 questions, evaluating 6 different domains (positive well-being, general health, depression, self-control,

anxiety and vitality). There are 6 possible answers for each question which are scored in a 0 to 5 scale. As with the AcroQoL, higher scores reflect more favourable HR-QoL outcomes [432].

2.3.5.iii. 36-item Short-Form (SF-36)

The SF-36 is a 36-item validated generic questionnaire to evaluate health status. It comprises of 8 scales which measure physical and mental health. The 8 sections are: physical function, role limitations due to physical health, role limitations due to emotional problems, energy/fatigue, emotional well-being, social functioning, pain and general health. Scores are transformed into a 0-100 scale, with 0 representing maximum disability and 100 representing no disability [433]. The total SF-36 score represents the average of the scores of the 8 subscales comprising the questionnaire.

2.3.5.iv. EuroQoL (EQ-5D)

The EQ-5D is another standardised generic questionnaire to measure health-related outcomes. The EQ-5D questionnaire consists of two parts: a 5-question assessment of patient's health status, with each question representing a different domain (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) and a visual analogue scale in which the patient is asked to evaluate their own health by giving a score on a 0-100 scale, with 0 being equivalent to worst health imagined and 100 equivalent to the best possible health. There are 3 possible answers for each of the 5 questions of the first part of the questionnaire, which are coded as a number 1-3, with 1 indicating absence of problems and 3 indicating presence of extreme/severe problem. The patient's health status is expressed as a 5-digit number based on the responses given, which is then converted to a score on -1 to +1 scale, with +1 representing best possible health and 0 representing death [434, 435].

2.3.6. Statistical analysis

Descriptive data are presented as mean and standard deviation, or medial and interquartile range for parametric and non-parametric data respectively. Non-paired t-test for continuous variables and Mann-Whitney U-test for variables which failed normality test were used to assess the difference in the QoL questionnaire scores between patients and controls at baseline. An additional comparison of the QoL scores was performed between patients with and without disease remission at baseline, using the non-paired t-test. For patients who completed the longitudinal arm of the study, the paired t-test was used to compare the baseline responses with those after the five-year follow-up period. Repeated-measures ANCOVA test, adjusting for several covariates, including age, gender, treatment modalities, duration of GH/IGF-1 control, GH/IGF-1 levels at the time of the QoL evaluation and presence of hypopituitarism, was also performed, in order to compare baseline and follow-up QoL scores. The Pearson and Spearman correlation tests were used to assess for correlations between continuous and non-continuous variables respectively. A multiple linear regression analysis was performed to assess for any variables which determine patients' QoL scores. A P value of <0.05 was considered statistically significant. Statistical analysis was performed using the statistics software "SigmaPlot" (Systat Software Inc. London, UK).

2.4. RESULTS

2.4.1. Cross-sectional arm

2.4.1.i. Patients' characteristics

Sixty-three patients with a history of acromegaly were approached to participate to the study. Questionnaires at baseline were completed by 58 patients (response rate 92%), 28 females and 30 males, with a mean age of 55.4±12.8 years. All patients previously received treatment for acromegaly; 50 patients (86.2%) had trans-sphenoidal surgery (TSS), 27 (48.3%) had cranial radiotherapy (cXRT; two patients had gamma-knife radiosurgery and the remaining had conventional external beam radiotherapy; mean XRT dose 45±0 Gy, mean number of fractions 25±0) and 34 patients (58.6%) received medical therapy with somatostatin analogues and/or dopamine agonists. Thirty-one patients (53.4%) were still on GH lowering medical therapy at the time of the QoL assessment (5 patients on oral dopamine agonists, 24 patients on slow-release somatostatin analogue injections and 2 patients on combined oral and injectable treatment). The mean interval from the initial treatment until the time of QoL evaluation at baseline was 11.6±8.2 years.

Mean values for GH and IGF-1 were 1.36±1.90 mcg/L and 101.2±52.7% of the upper limit of normal (ULN) respectively. Thirty patients (51.7%) were in remission based on the American Endocrine Society criteria; fifteen patients (25.9%) did not fulfill the disease remission criteria for both GH and IGF-1, whereas in thirteen patients (22.4%) there was discordance between GH and IGF-1 levels. The mean duration of GH and IGF-1 control was 5.1±5.1 and 4.1±4.7 years respectively. A summary of the patients' characteristics can be found in Table 2.1.

QoL was also assessed in 116 healthy controls, matched for age and gender with patients, at a ratio 2:1 (controls: patients). The control group included 56 females and 60 males (mean age 55.0±12.9 years).

2.4.1.ii. Quality of Life assessment

Patients previously treated for acromegaly demonstrated worse QoL compared with controls, with significantly lower median total scores in all QoL questionnaires; PGBWS: 72 (IQR 52-87) vs. 87 (IQR 77-94), p<0.001; SF-36: 57.6 (IQR 35.8-76.2) vs. 84.8 (IQR 74.1-90.5), p<0.001); EQ-5D: 0.69 (IQR 0.52-0.80) vs. 0.88 (IQR 0.73-1.00), p<0.001; and EQ-5D VAS: 66.5 (IQR 49.3-80.0) vs. 85.0 (IQR 70.0-90.0), p<0.001. With the exception of the PGWBS "Anxiety" subscale, in which median scores were not statistically different in the patient and control groups [19 (IQR13-22) vs. 20.0 (IQR 17.0-22.0) respectively, p=0.12], healthy volunteers had consistently better QoL outcomes in all subscales of the questionnaires used in this study.

The greatest differences in the QoL scores between patients and controls were noticed in the physical subscales of the questionnaires; "Positive well-being" and "General health" subscales for the PGWBS questionnaire; "Physical function", "Role limitations due to physical health", "Energy/fatigue" and "Pain" subscales for the SF-36 questionnaire. The magnitude of the differences in the emotional/psychological subscales, although statistically significant between patients and controls, with more favourable outcomes for the control group, was not as great as those observed in the physical subscales (Table 2.2).

Notably the QoL scores of the control population of this study were comparable with the previously QoL data for the UK population [491]. Table 2.3 provides a comparison of the SF-36 subscale scores between the individuals comprising the control group in this study and previously published UK population data.

Regarding the AcroQoL questionnaire, the domain of "Appearance" was the most affected in patients with acromegaly [median score 39% (IQR 29-62%)], followed by the "Physical" subscale [median score 47% (IQR 27-66%)]. Patients demonstrated higher median score in the "Personal relationships" subscale [70% (IQR 46-82%)].

A separate analysis of the QoL scores was performed between patients with biochemical disease remission (n=30) and those with elevated GH and/or IGF-1 values at the time of QoL evaluation (n=28), based on the clinical practice guidelines criteria by the American

Endocrine Society. A significant difference in GH [median GH 0.4 mcg/L (IQR 0.2-0.6 mcg/L) Vs. 1.8 mcg/L (IQR 1.1-2.5 mcg/L) respectively, p<0.001] and IGF-1 values [median IGF-1 69.7% ULN (IQR 51.3-86.7% ULN) Vs. 123.3% ULN (IQR 103.6-156.7% ULN) respectively, p<0.001] was noted; however, no difference in the QoL total and subscales scores in all questionnaires was found between the two subgroups (Table 2.4).

2.4.1.iii. Correlations

Duration of GH and IGF-1 control, female gender and increasing age were found to positively affect at least some aspects of patients' QoL. Duration of IGF-1 control was positively correlated with total and all subscale scores for AcroQoL, while duration of GH control was positively correlated with total AcroQoL (r=0.33, p=0.013), AcroQoL "Appearance" (r=0.35, p=0.007), AcroQoL "Personal Relationships" (p=0.305, p=0.02) and total PGWBS score (r=0.262, p=0.047). Female gender was associated with higher QoL scores in the AcroQoL "Physical" subscale (r=0.273, p=0.038), SF-36 (r=0.26, p=0.049), EQ-5D (r=0.472, p<0.001) and EQ-5D VAS (r=0.278, p=0.04).

Regarding negative factors affecting QoL, the use of GH-lowering therapy was negatively correlated with all AcroQoL subscales, as well as EQ-5D and VAS scores. Additionally, with the exception of AcroQoL "Appearance" subscale, LH/FSH deficiency was negatively correlated with all other aspects of AcroQoL questionnaire, as well as total scores for PGWBS, SF-36, EQ-5D and VAS score. Table 2.5 summarises the correlations found among the various acromegaly-related parameters and the QoL scores.

In the multiple linear regression analysis model, use of GH lowering therapy was found to be an independent negative predictive factor of the total AcroQoL score (coefficient -12.939, p=0.037) and the AcroQoL "Appearance" subscale score (coefficient -16.132, p=0.022); age was an independent variable, predictive of the total PGWBS score (coefficient 0.548, p=0.012) and EQ-5D VAS score (coefficient 0.617, p=0.034); while female gender was the only independent variable found to be predictive of EQ-5D score at the time of baseline assessment (coefficient 0.336, p=0.006).

2.4.2. Longitudinal arm

2.4.2.i. Patients' characteristics

Patients who completed the baseline QoL questionnaires prior to 2010 were invited for a second QoL evaluation, at least 5 years after the initial assessment. Of the 36 eligible patients, 28 patients completed the follow-up assessment (response rate 77.8%); of the remaining patients, 3 patients died, 1 was lost to follow-up and 4 patients declined. There was no difference in the patients' characteristics and baseline total scores for AcroQoL, PGWB, SF-36 and EQ-5D, between patients who completed the longitudinal arm of the study compared with those only assessed at baseline (Table 2.6).

Responses at baseline and follow-up were collected from 13 male (46.4%) and 15 female (53.6%) patients with a mean age of 56.1±10.3 years. The mean interval between the two time points of QoL evaluation was 5.7±0.6 years. 96.4% of patients had TSS, 57.1% received cXRT, and 53.6% received medical treatment. Thirteen patients (46.4%) were still on medical therapy at the time of the QoL evaluation at follow-up.

GH levels at baseline and follow-up were 1.58±2.32 mcg/L and 0.69±0.74 mcg/L respectively (p=0.002), while the IGF-1 values were 102.4±63.0% and 105.0±52.0% of ULN respectively (p=0.81). The mean duration of GH and IGF-1 control at baseline was 4.7±4.8 years and 4.1±4.1 years respectively, whereas at follow-up patients had a mean duration of 8.3±6.8 years of GH control and 7.8±7.4 years of IGF-1 control. Evidence of LH/FSH, ACTH, TSH deficiency and diabetes insipidus was present in 50%, 35.7%, 35.7% and 3.6% of patients respectively. Table 2.1 summarises the clinical characteristics of the patients who completed the longitudinal arm of the study.

No progression of the pituitary hormone deficits occurred between baseline and follow-up QoL evaluation. Three patients, who were treated medically, discontinued medical treatment and a further three patients had their dose reduced during the follow up period due to improved GH and IGF-1 control, while two patients had their medication dose increased in order to achieve a more optimum biochemical disease control. However, none of the patients

had additional interventions to the pituitary gland in terms of surgery or radiotherapy, between baseline and follow up assessment.

2.4.2.ii. Quality of Life assessment

No difference in the mean total and subscale scores of the AcroQoL, PGWBS, SF-36, EQ-5D and EQ-5D VAS score were observed between baseline and follow-up responses, with the exception of the SF-36 "Physical Function", in which a less favourable outcome was found at follow-up compared with baseline (58.5±24.7% vs. 43.1±31.1%; p=0.002). Following adjustment for covariates including age, gender, treatment modalities, hypopituitarism, duration of IGF-1/GH control and GH/IGF-1 levels at the time of the QoL evaluation, no difference among all baseline and follow-up QoL scores was observed, including the SF-36 "Physical Function" subscale. A summary of the patients' QoL scores at baseline and follow-up can be found in Table 2.7.

Further analyses were performed between patients with improved QoL scores at follow-up in comparison with the baseline values and patients whose QoL scores deteriorated at follow-up. When the minimally important difference (MID) was applied to define changes in the QoL scores between baseline and follow-up assessment, patients with an increase in the EQ-5D score (but not the VAS score) were found to have lower IGF-1 scores [median IGF-1 75.1% ULN (IQR 60.8-96.3% ULN)] at follow-up, but not at baseline, compared with the patients in whom deterioration in the EQ-5D score was noted [median IGF-1 128.1% ULN (IQR 100.5-153.7% ULN)], p=0.02. With regards to all other QoL questionnaires, no difference in terms of age, male/female ratio, duration of disease control, current GH levels, prevalence of hypopituitarism and proportion of patients who had radiotherapy or medical treatment was noted; however the number of patients fulfilling the criterion of the MID in their QoL scores was small (less than eight patients in each subgroup) and therefore the possibility of a type II statistical error cannot be excluded.

When any numerical change in the QoL scores between baseline and follow-up assessment was applied as criterion to define improvement or deterioration in QoL (i.e. increase or

decrease in the QoL scores of any degree), higher IGF-1 values at baseline were found in patients whose total AcroQoL scores declined between baseline and follow-up assessment, compared with patients in whom total AcroQoL scores increased [median IGF-1 at baseline 96.7 %ULN (IQR 78.9-160.5 %ULN) vs. 76.2 %ULN (IQR 45.0-95.1 %ULN) respectively; p=0.0034]. No other differences in any other patients' parameters were observed.

2.4.2.iii. Correlations

IGF-1 levels at the time of the follow-up assessment were negatively correlated with the AcroQoL "Physical" subscale scores (r= -0.415, p=0.03), but not with the total or the scores in the other two AcroQoL subscales (appearance and personal relationships). A positive correlation was also found between duration of IGF-1 control and AcroQoL "Physical" subscale scores (r=0.42, p=0.03). In a multiple linear regression analysis model duration of IGF-1 control was found to be an independent positive predictive factor of the total AcroQoL score (coefficient 1.904, p=0.05) and the AcroQoL "Personal Relationships" subscale score (coefficient 2.408, p=0.03), whereas duration of GH control was a positive predictive factor for the AcroQoL "Appearance" subscale scores at follow-up (coefficient 2.675, p=0.018).

Table 2.1.

Clinical characteristics of patients (mean ± SD, or %) who completed the quality of life questionnaires at baseline (N=58) and of those who participated to the longitudinal arm of the study (n=28; mean follow-up 5.7±0.6 years). *p-value of 0.002, ǂp-value of 0.81. A p-value of <0.05 was considered to be statistically significant. ACTH: adrenocorticotropic hormone; ADH: antidiuretic hormone; FSH: follicle stimulating hormone; GH: growth hormone; IGF-1: insulin-like growth factor 1; LH: luteinizing hormone; QoL: quality of life; TSH: thyroid stimulating hormone; ULN: upper limit of normal.

Characteristic	**Patients completed QoL assessment at baseline (N=58)**	**Patients completed baseline and follow-up QoL assessment (n=28)**
Gender		
• Male	30 (51.7%)	13 (46.4%)
• Female	28 (48.3%)	15 (53.6%)
Age (years)	55.4±12.8	56.1±10.3 (at baseline) 61.8±10.2 (at follow-up)
Treatment modalities		
• Trans-sphenoidal surgery	50 (86.2%)	28 (100%)
• Cranial radiotherapy	27(48.3%)	16 (57%)
• Medical therapy	34 (58.6%)	15 (53.6%)
Pituitary dysfunction following therapy		
• LH/FSH deficiency	25 (43.1%)	14 (50%)
• ACTH deficiency	22 (37.9%)	10 (35.7%)
• TSH deficiency	17 (29.3%)	10 (35.7%)
• ADH deficiency	2 (3.4%)	1 (3.6%)
Biochemical disease status		
• Duration of active acromegaly (years)	11.3±7.0	12.8±6.3
• Duration of GH control (GH <1 mcg/L) (years)	5.1±45.1	4.7±4.8 (at baseline) 8.3±7.8 (at follow-up)

• Duration of IGF-1 control (years)	4.1±4.7	4.1±4.1 (at baseline) 7.8±7.4 (at follow-up)
• GH (mcg/L)	1.36±1.90	1.58±2.32* (at baseline) 0.69±0.74* (at follow-up)
• IGF-1 (% ULN)	101.2±52.7	102.4±63.0%∓ (at baseline) 105.0±52.0%∓ (at follow-up)

Table 2.2. Quality of life assessment between patients previously treated for acromegaly (N=58) and age and gender matched controls (N=116) (median values with interquartile range in brackets). Higher scores represent better quality of life. A p-value of <0.05 was considered statistically significant. AcroQoL: Acromegaly Quality of Life questionnaire; EQ-5D: EuroQoL questionnaire; PGWBS: Psychological General Well-Being Schedule; SF-36: 36-item Short-Form; VAS: Visual Analogue Scale.

Questionnaire	**Patients** (N=58)	**Controls** (N=116)	**P-value**	**Score range (min-max possible score)**
AcroQoL				
Total score (%)	54 (36-65)	n/a	n/a	0-100%
• Physical (%)	47 (27-66)	n/a	n/a	0-100%
• Appearance (%)	39 (29-62)	n/a	n/a	0-100%
• Personal relationships (%)	70 (46-82)	n/a	n/a	0-100%
PGWBS				
Total score	72 (52-87)	87 (77-94)	<0.001	0-110
• Positive well-being	10 (7-14)	15 (12-16)	<0.001	0-20
• General health	8 (5-11)	12 (10-13)	<0.001	0-15
• Depression	12 (10-14)	14 (12-15)	0.001	0-15
• Self-control	12 (8-14)	14 (12-15)	<0.001	0-15
• Anxiety	19 (13-22)	20 (17-22)	0.12	0-25
• Vitality	10 (6-14)	14 (11-16)	<0.001	0-20
SF-36				
Total score (%)	57.6 (35.8-76.2)	84.8 (74.1-90.6)	<0.001	0-100%
• Physical function (%)	58.6 (34.6-85.0)	90.0 (71.3-100)	<0.001	0-100%
• Role limitations due to physical health (%)	29.2 (0-100)	100 (100-100)	<0.001	0-100%

• Role limitations due to emotional problems (%)	100 (33.3-100)	100 (100-100)	<0.001	0-100%
• Energy/fatigue (%)	40.0 (23.8-57.5)	70.0 (55.0-80.0)	<0.001	0-100%
• Emotional well-being (%)	80.0 (64.0-88.0)	88.0 (76.0-92.0)	0.001	0-100%
• Social functioning (%)	75.0 (50-100)	100 (75-100)	<0.001	0-100%
• Pain (%)	45.0 (22.5-70.0)	80.0 (67.5-90.0)	<0.001	0-100%
• General health (%)	43.8 (24.0-62.5)	66.7 (54.2-83.3)	<0.001	0-100%
EQ-5D				
Total score	0.69 (0.52-0.80)	0.88 (0.73-1.00)	<0.001	-1.00 to +1.00
VAS score	66.5 (49.3-80.0)	85.0 (70.0-90.0)	<0.001	0-100

Table 2.3. Comparison of the SF-36 subscale scores between the control group recruited in this study (N=116) and previously published UK population data (N=8889) [45]. Scores are shown as mean numbers with standard deviations.

SF-36 Subscale	**Kyriakakis et al. (N=116)**	**Jenkinson et al (N=8889)**
Physical Function	82.60±22.57	87.99±19.65
Role limitations due to physical health	87.18±26.94	87.17±22.01
Role limitations due to emotional problems	87.64±27.52	85.75±21.18
Energy/Fatigue	65.04±20.15	58.04±19.60
Emotional well-being	82.54±14.88	71.92±18.15
Social functioning	86.42±22.30	82.77±23.24
Pain	77.20±21.92	78.80±23.01
General health	67.01±19.39	71.06±20.43

Table 2.4. Comparison of the QoL scores across different questionnaires and subscales between patients who achieved disease remission following treatment for acromegaly (n=30) and patients who continue to have biochemical evidence of active acromegaly based on the American Endocrine Society criteria. Results are presented as median values with interquartile range (IQR) in brackets. The Mann-Whitney test was applied to assess for statistical differences in the median scores between the two groups. No significant differences in the QoL scores were found.

Questionnaire	**Patients with disease control**	**Patients with biochemically active disease**	**P-value**
ACRO-QoL			
Total score (%)	51.1 (28.1-64.8)	54 (37.5-68.1)	0.36
• Physical (%)	43.8 (25-63.3)	50 (36-70.3)	0.31
• Appearance (%)	42.9 (26.8-64.3)	39.3 (28.6-58.9)	0.76
• Personal relationships (%)	64.3 (37.5-82.1)	75 (53.6-83.9)	0.29
PGWBS			
Total score	67.5 (46-84.3)	73 (59.3-88.3)	0.27
• Positive well-being	9 (7-14.5)	12 (8.8-14.3)	0.25
• General health	8 (4.8-11)	8.5 (5-11)	0.62
• Depression	11.5 (6.8-14)	13 (11-15)	0.22
• Self-control	10.5 (8-14)	12.5 (9-14)	0.27
• Anxiety	18.5 (11.75-22)	19 (15.3-22)	0.38
• Vitality	9 (6-13)	11 (6.3-14)	0.25
SF-36			
Total score (%)	55.5 (30.2-77.3)	59 (40.6-76.7)	0.47
• Physical function (%)	60 (32.5-82.5)	57.1 (34.2-85)	0.54
• Role limitations due to physical health (%)	25 (0-100)	50 (0-100)	0.39

• Role limitations due to emotional problems (%)	100 (16.7-100)	66.7 (33.3-100)	0.42
• Energy/fatigue (%)	30 (20-60)	45 (25-60)	0.55
• Emotional well-being (%)	80 (48-90)	80 (72-88)	0.52
• Social functioning (%)	75 (43.8-100)	87.5 (59.4-100)	0.36
• Pain (%)	45 (22.5-73.8)	57.5 (22.5-70)	0.45
• General health (%)	41.7 (20.8-64.6)	50 (31.3-70)	0.31
EQ-5D			
Total score	60 (35.5-82.3)	69 (50-80)	0.29
VAS score	0.69 (0.34-0.8)	0.69 (0.52-0.8)	0.68

Table 2.5. Correlations between acromegaly-specific parameters and QoL scores at baseline. AcroQoL: Acromegaly Quality of Life questionnaire; ACTH: adrenocorticotropic hormone; cXRT: cranial radiotherapy; EQ-5D: EuroQoL questionnaire; FSH: follicle stimulating hormone; GH: growth hormone; IGF-1: insulin-like growth factor 1; LH: luteinizing hormone; NS: non-significant; PGWBS: Psychological General Well-Being Schedule; SF-36: 36-item Short-Form; TSH: thyroid stimulating hormone; TSS: trans-sphenoidal surgery; VAS: Visual Analogue Scale.

	AcroQoL				**Total PGWBS score**	**Total SF-36 score**	**EQ-5D**	
	Total score	**Physical**	**Appearance**	**Personal relationships**			**Total score**	**VAS score**
Age	NS	0.259 (p=0.049)	NS	NS	0.389 (p=0.003)	NS	NS	0.294 (p=0.03)
Female gender	NS	0.273 (p=0.038)	NS	NS	NS	0.26 (p=0.049)	0.472 (p<0.001)	0.278 (p=0.04)
Duration of active disease	NS	NS	NS	NS	NS	NS	NS	NS
TSS	NS	NS	NS	NS	NS	NS	NS	NS
cXRT	NS	NS	NS	NS	NS	NS	NS	NS
Medical therapy	-0.404 (p=0.002)	-0.34 (p=0.009)	-0.407 (p=0.002)	-0.265 (p=0.045)	NS	NS	-0.281 (p=0.03)	-0.337 (p=0.01)
Duration of GH control	0.33 (p=0.012)	NS	0.35 (p=0.007)	0.305 (p=0.02)	0.262 (p=0.047)	NS	NS	NS
Duration of IGF-1 control	0.325 (p=0.013)	0.296 (p=0.024)	0.288 (p=0.03)	0.309 (p=0.02)	NS	NS	NS	NS
Current GH	NS	NS	NS	NS	NS	NS	NS	NS
Current IGF-1	NS	NS	NS	NS	NS	NS	NS	NS
FSH/LH deficiency	-0.3988 (p=0.002)	-0.477 (p<0.001)	NS	-0.335 (p=0.01)	-0.34 (p=0.009)	-0.386 (p=0.003)	-0.364 (p=0.006)	-0.341 (p=0.01)
ACTH deficiency	NS	NS	NS	NS	NS	NS	NS	NS

Table 2.6. Comparison of the baseline patients' characteristics and quality of life scores among patients who completed the longitudinal arm of the study (n=28); and those who only participated in the cross-sectional arm (n=30); No differences were found between the two subgroups. AcroQoL: Acromegaly Quality of Life questionnaire; EQ-5D: EuroQoL questionnaire; GH: growth hormone; IGF-1: insulin-like growth factor 1; L: litre; mcg: micrograms; PGWBS: Psychological General Well-Being Schedule; SF-36: 36-item Short-Form; ULN: upper limit of normal; VAS: Visual Analogue Scale.

Characteristic	**Baseline QoL assessment only** (n=30)	**Baseline and Follow-up QoL assessment** (n=28)	**P-Value**
Age at baseline (years)	55.7±14.5	56.1±11.8	0.9
Male/Female ratio	17/13	13/15	0.6
GH at baseline (mcg/L)	1.2±1.5	1.51±2.31	0.5
IGF-1 at baseline (% ULN)	102.4±43.8%	102.4±63.0%	0.99
AcroQoL score (baseline)	54±23.3%	49.1±18.3%	0.4
PGWBS score (baseline)	69±24	66±21	0.6
SF-36 score (baseline)	46.3±25.4	43.0±23.7	0.6
EQ-5D score (baseline)	0.58±0.39	0.6±0.3	0.8
EQ-5D VAS score (baseline)	60±26	65.7±19.1	0.36

Table 2.7. Quality of life assessment results in patients with cured acromegaly (N=28) at baseline and follow-up (mean interval 5.68±0.6 years; mean ± SD). Higher scores represent better quality of life. AcroQoL: Acromegaly Quality of Life questionnaire; EQ-5D: EuroQoL questionnaire; PGWBS: Psychological General Well-Being Schedule; SF-36: 36-item Short-Form; VAS: Visual Analogue Scale.

Questionnaire	**Baseline assessment**	**Follow-up assessment**	**P-value (t-test)**	**P-value (repeated measures ANCOVA)**	**Score range (min-max possible score)**
AcroQoL					
Total score (%)	49±19	50±22	0.66	0.98	0-100%
• Physical (%)	44±21	44±24	0.93	0.76	0-100%
• Appearance (%)	39±20	44±27	0.32	0.42	0-100%
• Personal relationships (%)	64±26	65±26	0.67	0.49	0-100%
PGWBS					
Total score	66±21	65±19	0.61	0.46	0-110
• Positive well-being	11±4	10±4	0.74	0.27	0-20
• General health	8±3	7±3	0.10	0.74	0-15
• Depression	11±3	11±3	0.86	0.57	0-15
• Self-control	11±3	12±3	0.12	0.72	0-15
• Anxiety	17±6	17±5	0.71	0.40	0-25
• Vitality	10±4	9±4	0.16	0.45	0-20
SF-36					
Total score (%)	53.9±22.4%	49.0±23.9%	0.07	0.82	0-100%
• Physical function (%)	58.5±24.7%	43.1±31.1%	**0.002**	0.38	0-100%
• Role limitations due to physical health (%)	37.8±45.3%	35.7±42.7%	0.73	0.64	0-100%

• Role limitations due to emotional problems (%)	67.9±39.0%	63.6±45.3%	0.41	0.70	0-100%
• Energy/fatigue (%)	36.5±21.9%	35.5±24.0%	0.71	0.80	0-100%
• Emotional well-being (%)	70.4±22.2%	69.6±20.1%	0.76	0.89	0-100%
• Social functioning (%)	73.2±25.8%	66.1±28.2%	0.17	0.32	0-100%
• Pain (%)	44.7±24.5%	42.6±26.2%	0.34	0.97	0-100%
• General health (%)	43±23.7%	37.1±23.4%	0.07	0.34	0-100%
EQ-5D					
Total score	0.61±0.32	0.55±0.32	0.32	0.84	-1.00 to +1.00
VAS score	65.7±19.1	57.7±21.1	0.31	0.065	0-100

2.5. DISCUSSION

This was a two-arm study to assess HR-QoL outcomes in patients with treated acromegaly and long-term biochemically stable disease. The assessment was performed long after the patients' initial diagnosis and treatment (mean time 11.6±8.2 years) and after an at least 4-year mean period of both GH and IGF-1 control. The study showed that patients perceive a significantly impaired QoL in all subscales of the questionnaires used for the QoL evaluation in this study (with the exception of the PGWBS "Anxiety" subscale), compared with age and gender-matched controls. This is consistent with the results of previous cross-sectional studies, which also report QoL impairment in patients despite disease remission [107, 112, 439, 448, 485].

The subscale of "appearance" was the most affected domain in the AcroQoL questionnaire, similar to previous reports [430]. Regarding the SF-36 and PGWBS questionnaires, the physical subscales (i.e. physical limitations, energy, vitality, pain) were consistently more underscored compared with the emotional and psycho-social domains of these questionnaires, suggesting that acromegaly has a higher impact on patients' physical function than emotional and psychological well-being. This is likely to be related with irreversible complications of the disease such as arthropathy, which is the most common cause of morbidity in patients with acromegaly, affecting up to 77% of patients based on clinical criteria and patient-reported outcomes and up to 99% based on radiological criteria [109]. In fact, acromegalic patients with joint symptoms were found to have significantly lower QoL scores compared with patients without joint symptoms. This was primarily related to a negative influence of arthropathy on the domain of physical activities and functioning and to a lesser extent due to an impact on the psychosocial subscales of the questionnaires employed [107, 112].

The results of our study showed that factors associated with higher scores and better HR-QoL outcomes included duration of GH and IGF-1 control, female gender and older age. LH/FSH deficiency and long-term use of GH lowering medical therapy were correlated negatively with the QoL values in some questionnaires. In contrast with previous studies which did not demonstrate an association between QoL and biochemical disease status [439, 445, 460, 471,

482], the findings of this study showed a positive correlation between baseline AcroQoL scores and duration of GH and IGF-1 control; and between SF-36 scores and duration of IGF-1 control. Duration of IGF-1 control was found to be predictive of the total AcroQoL and "Personal Relationships" subscale scores at follow-up, while duration of GH control was predictive of the AcroQoL "Appearance" subscale score. These results suggest that duration of biochemical disease remission is an independent factor, which determines QoL in patients following treatment of acromegaly.

When patients who fulfilled the biochemical criteria for disease remission were compared with patients who were still showing evidence of biochemical disease activity at baseline, no difference was observed in the QoL scores between the two subgroups. However, patients who demonstrated a decline of their scores in some questionnaires, including the AcroQoL and the EQ-5D, during follow-up, had significantly higher IGF-1 values compared with patients in whom AcroQoL scores improved during the follow-up period. Therefore, it can be speculated that the biochemical disease status, although it may not be associated with the patients' current QoL as a numerical value, it may be predictive of the patients' QoL progression (improvement or decline).

The above suggest that early diagnosis of the disease and successful treatment in order to achieve rapid biochemical disease control could potentially lead to improved HR-QoL outcomes in the long-term. Minimising the duration of the total exposure to GH/IGF-1 excess may prevent patients from developing irreversible comorbidities associated with acromegaly, which may also influence QoL. Higher disease activity, as suggested by the higher pre-treatment GH and IGF-1 standard deviation scores (SDS), as well as greater GH exposure, has been associated with arthopathic changes (i.e. joint space narrowing) in the hips and knees [111], while patients with IGF-1 SDS in the highest tertile were found to have increased risk of developing osteoarthritis, particularly in the hip and hand joints [492]. Additionally, acromegaly has been associated with obstructive sleep aspnoea [183], which may also contribute to patients' impaired QoL and can persist in some patients even after biochemical remission of acromegaly [185, 192]. In the current cohort, despite the fact that

the patients had a more favourable biochemical status at follow-up with significantly lower GH levels compared with the baseline values, no improvement in the QoL scores was noted. In fact, the "physical function" subscale scores of the SF-36 questionnaire were significantly worse at follow-up compared with baseline, suggesting that the irreversible changes of acromegaly may develop relatively early in the disease course.

On-going use of GH lowering treatment was found to be a negative predictive factor of QoL, as assessed by the AcroQoL and EQ-5D questionnaires. This is in agreement with previous studies, which report that the need for prolonged treatment with somatostatin analogues to achieve biochemical control in patients with acromegaly following trans-sphenoidal surgery, has a negative impact on patients' QoL [471, 493]. A possible explanation for this finding is that patients who require long-term medical treatment may experience a higher disease activity, compared with patients who were cured following TSS. In this cohort, patients who were on long-term medical therapy had significantly higher IGF-1 levels and shorter duration of biochemical disease control, compared with patients not on somatostatin analogues or dopamine agonists, suggestive of a more resistant-to-treatment disease in the former subgroup of patients. Additionally, the need for regular somatostatin analogue injections may cause an additional psychological distress to patients and act as a continuous reminder of their disease, which may also contribute to patients' impaired QoL and perceived well-being.

In contrast with previous reports [471], the results of this study showed that female gender was associated with better QoL outcomes, particularly for the AcroQoL "physical" subscale, EQ-5D and EQ-5D VAS scores. Assessing other parameters between the two gender groups, a difference in the disease activity biochemical markers was found. Female patients had lower IGF-1 levels at baseline compared with male patients (89.2±46.5 %ULN vs. 122.0±69.5 %ULN respectively, p=0.04) with longer duration of IGF-1 control (4.8±4.3 years vs. 3.0±4.3 years respectively, p=0.056). Therefore, the gender-related differences observed in the QoL scores, may reflect the different biochemical disease states in the two gender groups. No difference between male and female patients was identified regarding patients' age, GH levels and duration of GH control.

Previous QoL studies in patients with acromegaly have shown a negative or no association between older age and QoL [107, 439, 471, 482]. However, this study showed a positive effect of increasing age on several QoL aspects (total PGWBS, EQ-5D VAS and AcroQoL "Physical" subscale). No similar association was observed in the control group. A possible explanation for this paradoxical finding could be that elderly patients may be more receptive to the diagnosis of a chronic disease, its complication and the need for invasive and/or long-term medical treatment, compared with patients of a younger age, who may have higher expectations of their general health and therefore perceive more impaired QoL. Ageing has been associated with a decline in QoL in previous population studies [494]; however, the results of a longitudinal study, performed on a large sample of non-institutionalised adults aged >50 years living in England, have shown that when controlling for gender, education, socio-economic status and long-term illnesses, QoL starts to improve after the age of 50, peaking at the age of 68, with a gradual decline thereafter [495], which is in support with the findings of this study.

Gonadotrophin deficiency was found to negatively influence QoL outcomes in all four questionnaires used in this study, despite the fact that patients were on stable hormone replacement therapy at the time of QoL evaluation. Previous studies have not shown any association between QoL and hypopituitarism following treatment for acromegaly [439, 459, 482]. However, a cross-sectional study by Tiemensma et al. showed that patients who were on hormone replacement therapy for hypopituitarism post treatment for acromegaly perceived less treatment control compared with patients with normal pituitary function [448]. Another possible explanation for the negative impact of gonadotrophin deficiency on QoL outcomes is via the effect of radiotherapy. Most cases of LH/FSH deficiency in this cohort were found in patients who previously received cranial radiotherapy. Although radiotherapy was not found to be a predictive factor for QoL in this study, results from other research groups report that cranial radiotherapy is an independent negative predictor of QoL [107, 460, 482].

Limitations to the study include the relatively small number of participants; however this is an issue commonly encountered in clinical studies of rare diseases. The patients were recruited

from a single tertiary pituitary centre. The study benefited from a high response rate (92% of the eligible patients completed the questionnaires at baseline), however a limiting factor was the total number of patients with acromegaly under the care of the single pituitary centre. A multi-centre study may have been able to address the sample size issue. Additionally, the control population comprised of patients' relatives and hospital staff. Regarding hospital staff recruitment, although individuals from a range of professional backgrounds were recruited, there is a possibility of selection bias. However, comparing the QoL scores from the control group of this study with previously published UK population data, particularly for the SF-36 questionnaire [491], no significant differences were observed (Table 2.3).

2.6. CONCLUSIONS

Based on the results of this study, it is evident that achieving and maintaining biochemical control in acromegaly does not translate in normalisation of QoL as this is perceived by patients. The patients in this cohort, despite having a more favourable biochemical profile at the time of their follow-up QoL evaluation, with significantly lower GH levels compared with the baseline assessment, they demonstrate no change in their QoL scores, which remained significantly impaired compared with age and gender-matched controls. The dichotomy between physical and psychological impairment in patients with acromegaly, as demonstrated by the QoL subscale scores of the questionnaires used in this study, suggests that apart from maintaining biochemical disease remission, clinicians' efforts should also be focused on the prevention and management of comorbidities, which impact on patients' physical function, as this has been shown to be the most significantly impaired QoL domain in patients with long-term disease control.

In conclusion, the results of this study confirm that patients with acromegaly demonstrate severely impaired quality of life, which persists in the long-term despite multimodality therapy and biochemically stable disease. This study is only the second prospective study to assess QoL in treated patients with long-term biochemical control of acromegaly, and benefits from the longest duration of follow-up reported in the literature (mean follow-up 5.7±0.6 years). Appearance and physical function are the domains in the QoL assessment tools affected to the greatest degree, which likely results from the irreversible skeletal and soft tissue changes that occur during the course of acromegaly. The skeletal changes that lead to arthropathy, which consists the commonest cause of morbidity in patients with acromegaly, are explored further in Chapter 3.

In addition to the physical complications of the disease, it is also important to highlight that patients with acromegaly demonstrate impaired emotional well-being and psychosocial functioning, which also persist in the long-term. Duration of GH and IGF-1 control, the biochemical disease status and the on-going use of GH lowering treatment at the time of the QoL evaluation correlated with QoL outcomes across the various questionnaires. Therefore,

early diagnosis and effective treatment to minimise the duration of active disease may contribute to patients' sustaining improved HR-QoL in the long-term. Additionally, a more holistic approach is required for the follow-up of patients previously treated for acromegaly, in which the focus of clinical care would be not only on aggressive biochemical disease control, but also on managing the comorbidities associated with acromegaly, including patients' psychosocial health, as both aspects impact on patients' perceived HR-QoL outcomes.

CHAPTER THREE

CHARACTERISING THE ARTHROPATHY IN ACROMEGALY: A LITERATURE REVIEW

3.1. ABSTRACT

Patients with acromegaly, even after long-term remission, are often left with chronic adverse sequelae and impaired quality of life. Among the various complications of acromegaly, arthropathy is the leading cause of morbidity, as reflected in multiple patient reported outcome studies. Undoubtedly, arthropathy is the biggest burden in acromegalic patients with controlled disease and remains relatively under-recognised and under-studied. The current thesis chapter summarizes the available literature on acromegalic arthropathy, focusing on the pathophysiological mechanisms responsible for the development of the arthropathy in people with acromegaly; the imaging features based on different anatomical areas of the skeleton that make this type of arthropathy unique and distinct from osteoarthritis; and factors specific to acromegaly that have been identified to be associated with increased risk of developing arthropathy or with increased severity of arthropathy. The chapter also covers the reported natural course of acromegalic arthropathy, the effect of medical treatment for acromegaly on structural joint alterations and finally the impact of arthropathy on patients' quality of life.

3.2. INTRODUCTION

Patients with acromegaly demonstrate impaired quality of life (QoL) as shown in a variety of cross-sectional studies [107, 112, 439, 448, 485]. This is also evident by the results of the cohort study presented in Chapter 2 of this thesis, in which patients with long-term acromegaly remission, who were followed up for a mean 5.7-year period, were found to have impaired QoL, which remained unchanged from the baseline assessment. These data suggest that successful treatment and biochemical disease control does not necessarily translate into normalisation of the patients' QoL.

Arthropathy is undoubtedly the commonest cause of morbidity in patients with long-standing acromegaly, affecting up to 77% of patients based on clinical criteria and patient-reported outcomes [107, 108] and up to 99% based on radiological criteria [109]. It is also one of the most important factors affecting patients' QoL in the long-term. Worse QoL outcomes were found in acromegalic patients with joint symptoms compared with patients without arthralgia [107, 112].

Arthropathy remains one of the unresolved issues amongst patients with acromegaly, even in those patients who have achieved biochemical disease remission following treatment. At present there are no established strategies for the management of this common complication. Efforts have been focused on developing therapeutic interventions to optimise biochemical control by normalising GH and insulin-like growth factor type 1 (IGF-1) levels, thereby minimising the excess mortality and morbidity. Despite the advancements in treatment based on biochemical control, joint-related symptoms appear to persist. The aim of this work is to comprehensively review all the currently available data about the pathophysiological mechanisms related to the development of the arthropathy in patients with acromegaly; the features of the acromegalic arthropathy on imaging; determine the factors that may increase the risk of a patient developing arthropathy; characterise the natural history of the acromegalic arthropathy; describe the effect of treatment on joint alterations; and finally evaluate the impact of the arthropathy on patients' QoL.

3.3. THE ARTHROPATHY IN ACROMEGALY – LITERATURE REVIEW

3.3.1. Pathophysiology

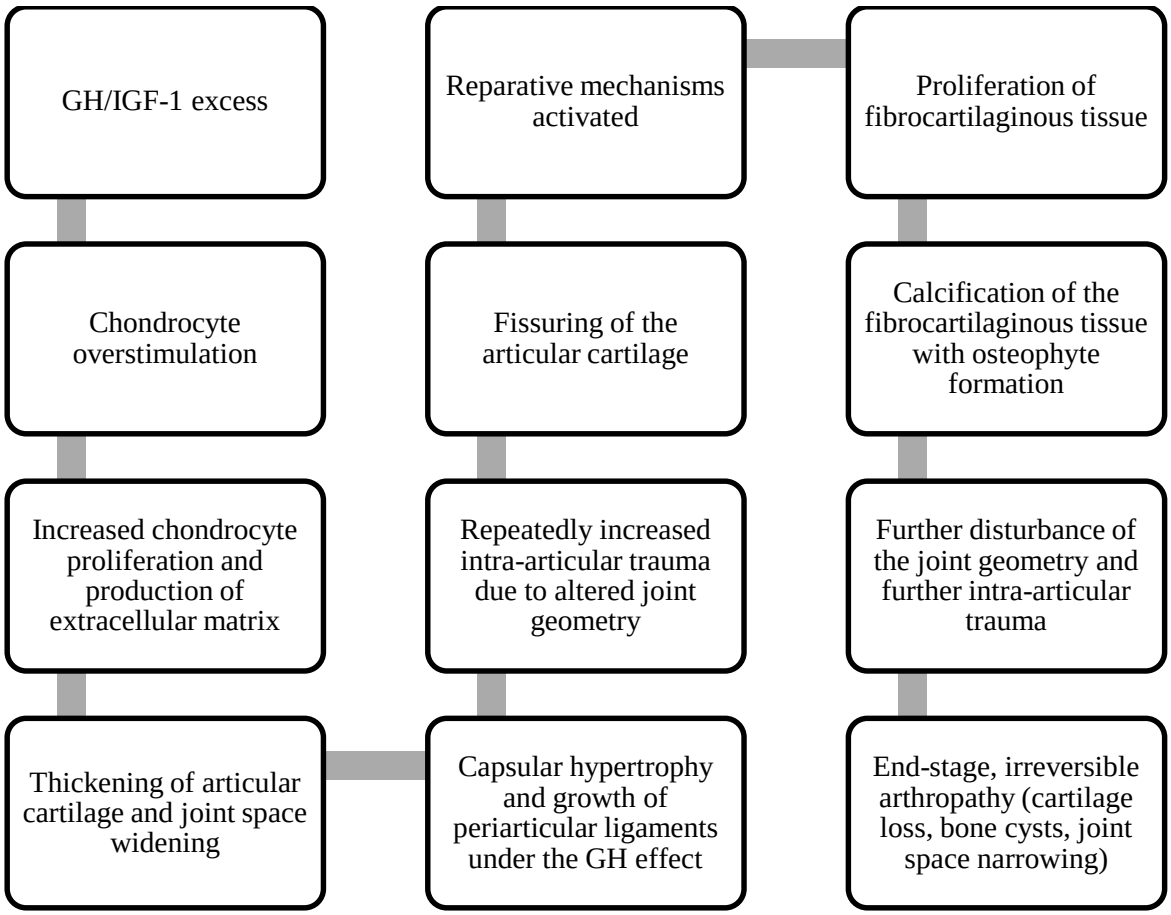
The pathophysiology behind the arthritic changes in acromegaly is yet to be fully elucidated. Previous studies have shown that GH has a stimulating effect on chondrocytes, especially promoting differentiation of the prechondrocytes from the resting cell layer and increasing their sensitivity to IGF-1 [496, 497]. IGF-1 is synthesized by chondrocytes, then acts in a paracrine/autocrine fashion [498, 499], stimulating DNA synthesis, cell proliferation and production of proteoglycans and glucosaminoglycans by chondrocytes [500-502]. Similar to the trophic effect of GH on chondrocytes, GH stimulates IGF-1 mRNA and peptide production by osteoblasts [503], promoting cell proliferation and production of collagen and alkaline phosphatase by osteoblasts [244, 504]. Apart from the autocrine/paracrine effect of the locally produced IGF-1, circulating IGF-1 has also been shown to regulate osteoblast metabolism [505]. The role of GH and IGF-1 in the articular changes that occur in acromegaly has been investigated in an animal model of acromegaly, in which the thighs of rats were inoculated with GH-secreting tumour cells. After 4 weeks of inoculation, multi-layered synovial and perichondral cells near the periarticular cartilage, positive for proliferating cell nuclear antigen and expressing IGF-1 mRNA, were detected. IGF-1 receptors were also present on these cells [506]. After 8 weeks of inoculation new bone and cartilage formation was detected near the articular cartilage, with expression of IGF-1 mRNA and IGF-1 receptors by the proliferating chondrocytes and osteoblasts. In parallel with these changes, an increase in the body weight and serum GH levels of the rats was noted [506].

Based on the above, a two-stage pathophysiological mechanism for the development and progression of the acromegalic arthropathy has been proposed. Initially, and under the direct effect of chronically elevated of GH and the IGF-1, there is overstimulation of chondrocyte proliferation and function, with increased production of extracellular matrix, leading to thickening of the articular cartilage and widening of the joint space, a common radiographic sign of joint imaging in patients with acromegaly. Additionally, GH promotes capsular

hypertrophy and growth of periarticular ligaments, causing laxity and hypermotility of the joint. This is the first stage in the development of the acromegalic arthropathy, characterized by overgrowth of the articular cartilage and periarticular soft tissues. It has been proposed that these changes are potentially reversible with successful treatment of the acromegaly [123, 507]. The patients tend not to be overtly symptomatic at this stage, due to the preserved or increased cartilage and joint space width.

During the second stage of the acromegalic arthropathy pathophysiological process, all the above changes alter the geometry of the joint, while abnormal mechanical joint loading results in repeated intra-articular trauma. As the arthropathy progresses, fissures develop on the articular cartilage surface. Simultaneously, reparative mechanisms are activated, as in osteoarthritis, leading to fibrocartilaginous tissue proliferation which occurs in an erratic fashion, possibly under the continuous effect of the elevated GH. This new fibrocartilaginous tissue often becomes calcified, forming osteophytes and exacerbating further the altered articular geometry and trauma. Cartilage fissures deepen (and may extend to the subchondral bone) causing cartilage ulcerations. In the late stages of the acromegalic arthropathy there is articular cartilage loss detected as joint space narrowing, particularly in the weight-bearing joints, whilst the subchondral bone shows increased turnover, eburnation and cyst formation, changes similar to primary osteoarthritis. These changes tend to be irreversible and normalization of GH and IGF-1 at this stage does not influence the already established arthropathy [123, 507, 508]. Figure 3.1 illustrates the proposed model for the different stages of the acromegalic arthopathy, from the early articular changes until the development of established joint disease.

Figure 3.1. Illustration of the proposed model for the different stages of the acromegalic arthopathy, from the early articular changes until the development of established joint disease.



3.3.2. Prevalence of Arthropathy in Acromegaly

The prevalence of arthropathy varies in the different studies depending on the criteria used to define 'arthropathy'. Overall patients with acromegaly appear to have a 4-12 fold higher risk of developing arthropathy than the background population [112].

No correlation has been demonstrated between patient reported outcomes and radiological abnormalities [112, 509]. Based on radiological criteria, arthropathy has been found to be most prevalent in the spine (92% in the cervical spine, 88% in the lumbar spine) [109], followed by the small joints of the hands in up to 68% of patients [112]. Hip and knee joints were affected in 29% and 36% of patients respectively [109], while shoulder joints were affected in 33-65% of patients [509, 513]. However based on patients' self-reported symptoms, the prevalence of arthropathy has been estimated at 61-65% in the spine [109, 112], 81% in the hands [109], 46-53% in the knees [109, 510], 13-58% in the hips [109, 510] and 35% in the shoulders [510]. Overall, arthropathy based solely on radiological criteria, present in at least one joint site, has been reported in up to 99% of patients with acromegaly [109], whereas clinical arthropathy based on patient-reported symptoms in at least one joint site has been reported in up to 77% of patients [107, 108]. The results of the above studies are based on cohorts of 58-89 patients with acromegaly [107-109, 112, 510], following adjustment for age, gender and BMI in some studies [107-109]. Above findings are summarized in Table 3.1.

Table 3.1. Prevalence of arthropathy in different anatomical sites in patients with acromegaly, based on radiological and clinical criteria.

Anatomical site	**Prevalence of arthropathy based on radiological criteria**	**Prevalence of arthropathy based on clinical criteria**
Spine	88-92%	61-65%
Hands	68%	81%
Hips	29%	13-58%
Knees	36%	46-53%
Shoulders	33-65%	35%
Any joint	99%	77%

3.3.3. Imaging Data

Acromegalic arthropathy has been considered as a non-inflammatory arthropathy [511], affecting joints of both the axial and appendicular skeleton [511-513]. In some cases, the acromegalic arthropathy may progress to a debilitating pathology with patients requiring joint replacement surgery for symptomatic relief [511]. Data on joint imaging in acromegaly are mainly derived from X-ray studies, while ultrasound scan has been applied in a small number of studies, computerised tomography in one case series and magnetic resonance imaging in one case-control study.

3.3.3.i. Appendicular skeleton - lower limbs

Preserved joint space or joint space widening (JSW) with presence of osteophytes is the commonest radiological feature in the large joints of the lower limbs in patients with acromegaly [108, 109, 111, 509, 511-517]. Joint space narrowing (JSN), the cornerstone feature of osteoarthritis (OA), was found to be present in only 10.3% of patients in the hip joints and in 15.4% in the knee joints [111]. Johanson et al in a series of 6 patients with acromegaly found JSN to be present in 4 patients; however this series only included patients who required total hip joint replacement, suggesting a selection bias [514]. Other abnormal findings from the radiological assessment of hip joints include enthesopathy [509], cartilage loss with sclerosis of subchondral bone and cyst formation [512] and cortical irregularity of the femoral head and acetabulum, consisting of erosive lesions of the subchondral bone [514]. Additional, but less common features of the acromegalic arthropathy in the knee joints include cartilage calcifications [509, 512, 513], capsular ossifications [512], enthesopathy with involvement of the tibial spines and the patellar insertion site of the quadriceps tendon [509, 513], coarsening of the bony trabeculae with widening of the distal femur and proximal tibia [511], synovitis with effusions varying in degree [511, 512, 517] and thickening of the prepatellar bursa [517]. Figure 3.2 provides an example of the radiographic changes in a hip joint in a female patient with advanced arthropathy, while figure 3.3. provides a radiographic illustration of a knee joint showing marked osteophytosis in a female patient with difficult to

treat acromegaly and history of bilateral hip joint replacements due to advanced arthropathy. Radiographic images were obtained from patients with acromegaly treated at Leeds Teaching Hospitals NHS Trust and were performed as part of their clinical care.

Figure 3.2. Pelvic x-ray performed in a 49-year-old female patient diagnosed with acromegaly at the age of 29 years and required multi-modality treatment with two pituitary surgeries, pituitary radiotherapy and medical treatment with octreotide LAR. The x-ray shows considerable bilateral hip joint degeneration, with thinning and irregularity of both hip joint spaces (red arrows), acetabular new bone formation and bilateral subchondral cystic changes in both femoral bones (blue arrows).

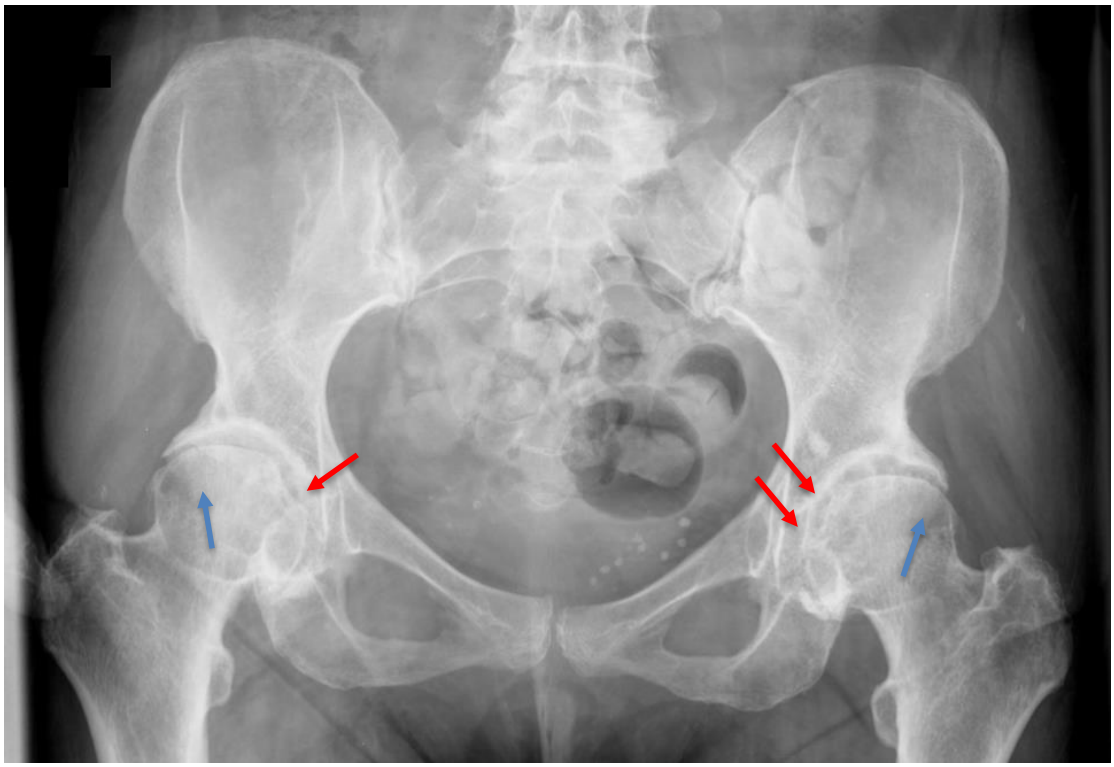
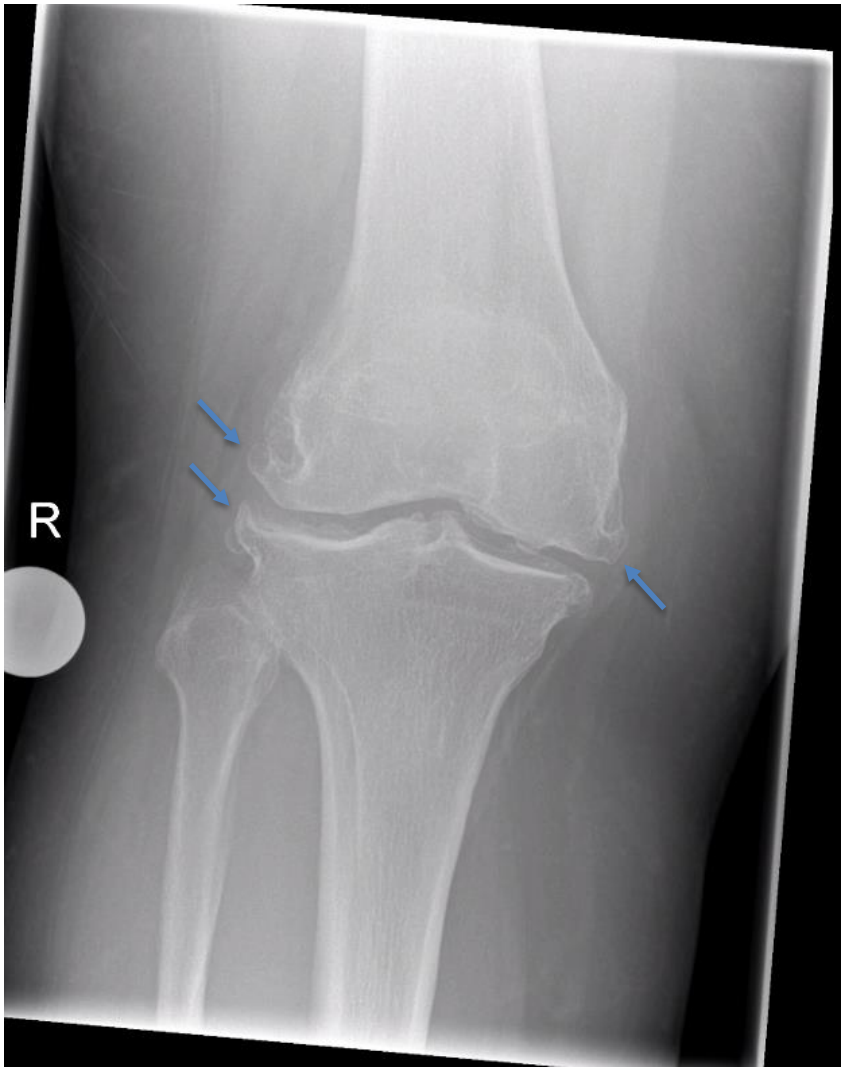


Figure 3.3. Right knee joint x-ray in a 32-year-old female patient, diagnosed with acromegaly at the age of 20 and underwent treatment with three pituitary surgeries, pituitary radiotherapy and octreotide LAR. Despite her young age the patient had also undergone bilateral hip replacements due to advanced arthropathy. The x-ray showed tri-compartmental degenerative disease with marked marginal osteophytosis (blue arrows).



Ultrasonographic studies of the knees have consistently shown that patients with active acromegaly have significantly increased thickness of articular cartilage compared with controls [518-521]; there are limitations, however, in how well ultrasound may quantify cartilage in joints where it does not have a good acoustic window, including the knee joint. When patients with active acromegaly were compared with patients in remission, mixed

results were reported: higher articular cartilage thickness in active acromegaly was reported by Colao et al in 3 studies [518-520], though no difference was seen in a case-control study [521].

In the only MRI study to date, several structural abnormalities (typical of OA) were identified in knees including osteophytes, bone marrow lesions and subchondral cysts, with similar prevalence in patients with active and controlled acromegaly [522]. This study reported greater cartilage thickness in patients with active acromegaly compared with patients in remission. Patients with active acromegaly also had evidence of abnormal biochemical cartilage composition, with higher T2 relaxation times than patients with controlled disease. The reproducibility of the T2 relaxation time measurements was reported as moderate with an intraclass correlation coefficient (ICC) of 0.53 [522]. When compared with controls comprising of patients with primary OA from previous studies, patients with acromegaly had lower rates in the prevalence of bone cysts and bone marrow lesions, without significant difference in cartilage defects and osteophytosis [522]. Additionally, patients with acromegaly demonstrated higher cartilage thickness and higher cartilage T2 relaxation times compared with primary OA patients, with the authors suggesting the presence of qualitative differences in the cartilage biochemical composition between the two diseases [522]. However there were several limitations to this study including the small number of patients with acromegaly (N=26); the higher proportion of female subjects in the OA group compared with the acromegaly group; and the use of reference data on cartilage T2 relaxation time in OA patients from the literature [522].

Radiographic examination of the ankle joints was available in one cohort study of 36 patients with acromegaly, whose disease status was not specified. The study reported mild structural changes, with marginal osteophytosis of the distal tibia and talus and no JSN [511].

The joints of the foot and the heel tendon thickness have been assessed in a number of clinical studies. Remodeling of the metatarsals, with narrowing of the shafts and thickening of the trabeculae at the bone ends, leading to separation of the metatarsal heads has been described based on radiological findings [517, 523]. CT imaging of the feet, in a case series of three

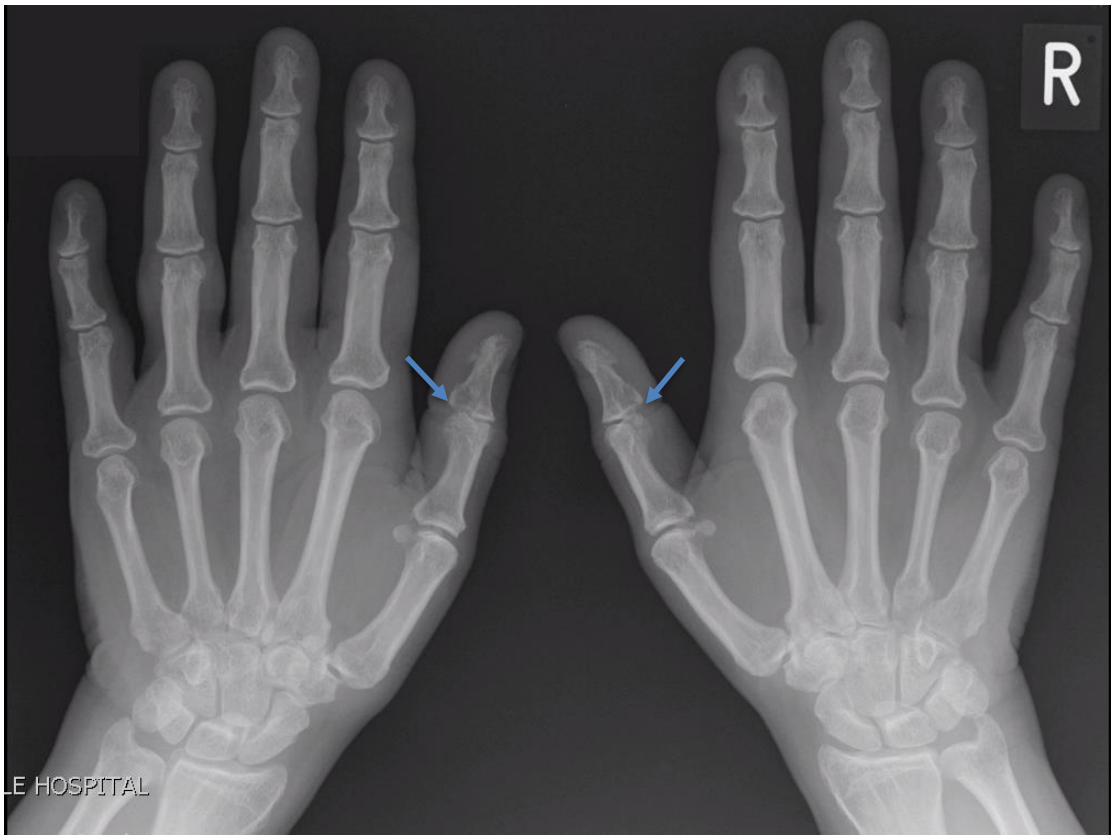
patients with acromegaly, demonstrated metatarsal penciling with V-shape deformity of the metatarsals due to bone resorption of the plantar cortex, separation of the metatarsal heads and shafts as well as soft tissue thickening at the plantar aspect of the foot [52]. Increased heel tendon thickness in patients with acromegaly compared with healthy controls and patients with inactive acromegaly was observed on ultrasonographic studies, however no information was provided regarding qualitative features of the thickened tendons (i.e. whether this was related to increased extracellular matrix or to inflammation/enthesitis) [518, 519].

3.3.3.ii. Appendicular skeleton - upper limbs

Similar to the joints of the lower limbs, JSW and osteophytosis are the main radiographic features of the arthropathy affecting the small joints of the hand, as well as the shoulders [509, 512, 513, 517, 525-527] in patients with acromegaly. Biermasz et al. in a case control study applied a semi-automatic quantitative method developed by van't Klooster [528] in order to measure joint space width of the hand joints in acromegalic patients. Increased joint space width was found in 89 patients with acromegaly when compared with 471 controls, in all joint areas of the hand, including the metacarpophalangeal (MCP) joints, the proximal interphalangeal (PIP) joints and the distal interphalangeal (DIP) joints, after adjusting for age and gender [529]. Other radiological abnormalities identified in the hand joints based on observational studies include osteophytes [530], particularly at the base of distal phalanges [512], bone remodeling of the metacarpals with thickening of the trabeculae in the bone-ends and narrowing of the shafts [517] and the phalanges (with loss of the waist at the distal part of the shaft and phalangeal squaring) [509, 512], squaring of the metacarpal bone heads because of exostosis [512, 527, 531], tufting of the distal phalanges [509, 512, 527, 530], enthesopathy of the proximal phalanges [509, 527], calcifications or ossifications of the joint capsule [512, 527] and increased sesamoid index (calculated by multiplying the greatest diameter of the medial sesamoid bone at the first MCP joint by the greatest diameter of the same sesamoid bone, perpendicular to the first diameter) [500, 525-527, 532, 533]. Figure 3.4 provides an example of early degenerative changes at the interphalangeal joint areas in a

female patient with acromegaly, who is under follow-up at Leeds Teaching Hospitals NHS Trust and the x-ray was performed for clinical purposes.

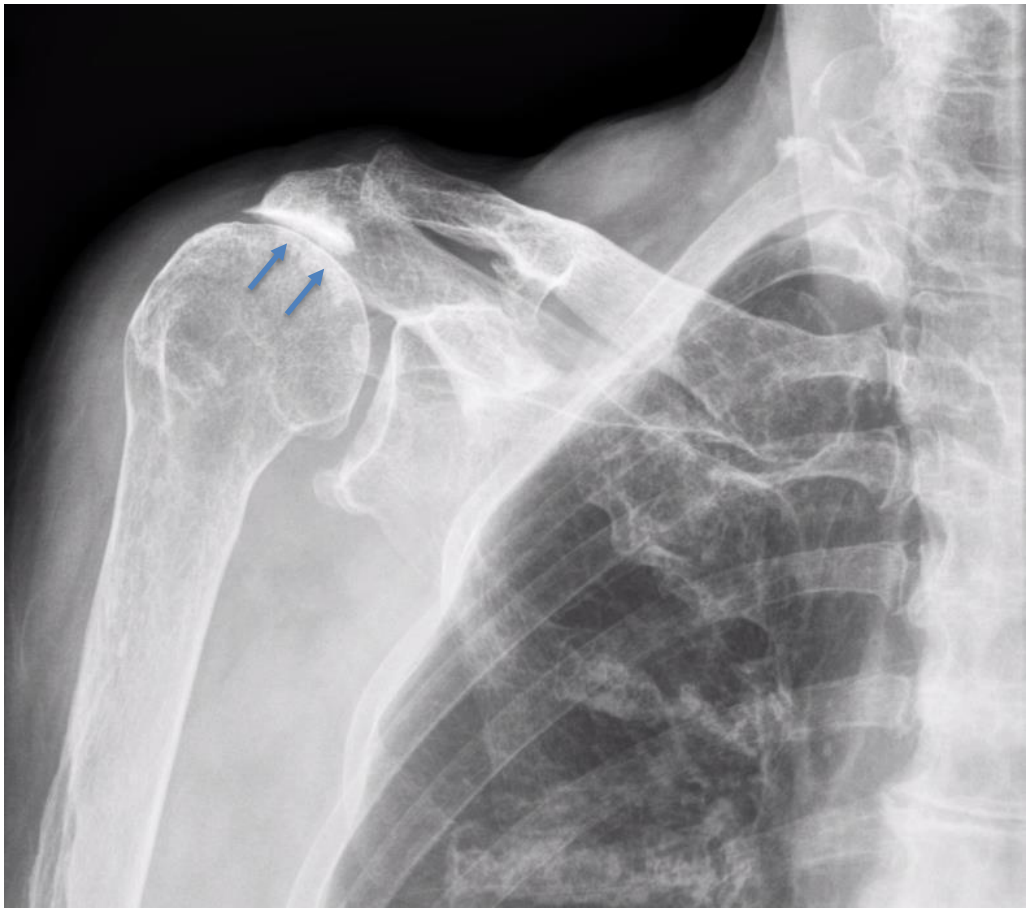
Figure 3.4. Bilateral hand x-rays in a 50-year-old female patient diagnosed with acromegaly at the age of 30 years and treated with one pituitary surgery, radiotherapy and octreotide LAR. The x-ray showed early features of osteoarthritis in the proximal interphalangeal joints of both hands and joint space narrowing in the interphalangeal joints of both thumbs (blue arrows).



When assessing arthropathy of the shoulder, JSW of the glenohumeral joint was the commonest finding, seen in 65% of patients in a cross-sectional study by Podgorski et al, followed by osteophytosis and enlargement of the distal clavicle (present in 47% of cases) [509]. JSN has also been described [513], however it appears to be a rather uncommon finding [511]. Cystic changes in the head of the humerus; osteocartilaginous proliferation across the inferior humeral head and the glenoid rim [511]; enthesopathy affecting the greater

tuberosity in 41% of cases and occasionally the clavicle at the coracoclavicular ligament attachment site [509] and thickening of the subacromial bursa [517], however these results are based on small cohorts with 17-36 patients. Figure 3.5 provides a radiographic illustration of shoulder arthropathy in a female patient with acromegaly who has been under the clinical care of Leeds Teaching Hospitals NHS Trust.

Figure 3.5. Right shoulder x-ray in a 49-year-old female patient, who also had pelvic x-ray, presented in Figure 3.2, showing pronounced upward subluxation of the humeral head with subacromial sclerosis (blue arrow), typical of rotator cuff degeneration. The joint space is preserved.



The elbows appear to be less commonly affected compared with other joint sites in acromegaly and the changes that occur seem to be less extensive. Detenbeck et al have reported elbow involvement in only 3 of 36 patients in their cohort, with the main radiological

abnormalities being degenerative hypertrophic changes with occasional loose bodies. The joint space was overall preserved, but no joint space widening has been reported [511]. Thickening of the olecranon bursa has been reported by Kellgren et al [517].

Ultrasonography of the wrists and shoulders has shown increased articular cartilage thickness in patients with active acromegaly, as well as patients with disease control, compared with healthy controls [518-520].

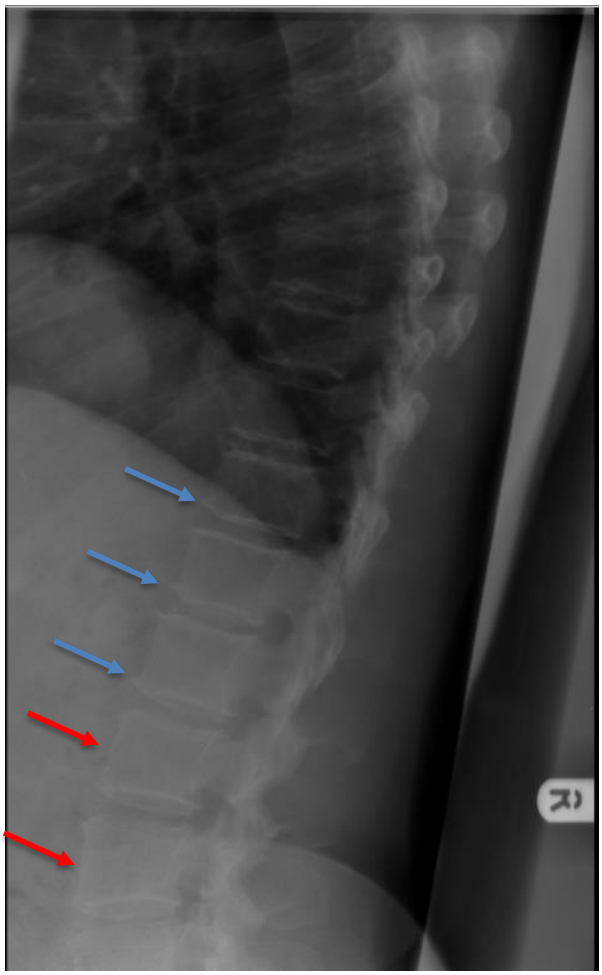
3.3.3.iii. Axial skeleton

Radiological alterations have been reported in all spinal regions including cervical, thoracic and lumbosacral tracts [110]. Common findings are kyphosis predominantly of the thoracic spine [110, 512, 517, 523]; osteophytes [509, 512, 513, 523] affecting all spinal regions [509], but most commonly the anterior aspects of the thoracic and lumbar vertebrae [512]; and increased vertebral dimensions with increased anteroposterior diameter [110, 509] and vertebral height [110, 523]. Tall but narrow vertebrae were found in 34% of females and 12% of males in a cross-sectional study of 104 acromegalic patients [523], however the authors did not provide a definition of 'tall' vertebrae and did not include a control group. In the same study the authors reported that broad and short vertebrae (which was also not defined) were found in 15% of females and 13% of males, and were only present in patients with inactive disease and resultant hypopituitarism [523].

The intervertebral disc space (measured at the L2-L3 level) was found to be reduced in 37% of patients with acromegaly in a case-control study; however when this was compared with controls, the difference did not achieve statistical significance ($p=0.051$) [110]. Widening of the disc space was less prevalent (present in 13% of the acromegalic patients); however no cases of disc space widening were observed in the control group [110]. Disc space narrowing, but not widening, was described in 2/19 patients with active acromegaly in a cohort study by Layton et al [513]. In contrast, in a cross-sectional study of 42 patients with active disease, Bluestone et al. reported increased disc space in the lumbar spine was a common finding, without providing a specific value for its prevalence [512]. Additionally, thickening of

intervertebral discs mainly in the lumbar spine was reported in another cross-sectional study of 104 patients with acromegaly, however without specifying the exact prevalence of this finding [523]. However, in both these studies [512, 523], data were presented in a descriptive way, without the use of control group or a standardized method for defining abnormal disc space or thickening. Figure 3.6 provides an example of some of the pathological findings described above based on a thoracolumbar spine x-ray of a female patient with acromegaly from Leeds Teaching Hospitals NHS Trust.

Figure 3.6. Thoracolumbar spine x-ray in a 49-year-old female patient, who also had x-rays of the pelvis and right shoulder, presented in Figures 3.2 and 3.5 respectively, showing scoliosis of the thoracic spine, hyperlordosis of the lumbar spine, presence of osteophytes in the anterior aspect of lumbar vertebrae (blue arrows), which appear tall (red arrows) and with preserved intervertebral disc space.



Other pathological findings reported from radiological examination of the spine include posterior scalloping of the vertebral bodies of the lower thoracic and lumbosacral spine (referring to concavity to the posterior aspect of the vertebral bodies when viewed from the lateral projection) [513, 523, 534]; linearization and hyperlordosis of the cervical and lumbosacral spine (prevalence not statistically different from controls) [110]; scoliosis of the thoracic and lumbosacral spine (though again prevalence was not statistically different from controls) [110]; ossification of anterior aspects of the discs; calcification of areas near the anterior margin of vertebral bodies [512]; periosteal appositions (predominantly in males) in the lower thoracic spine, with the lumbar spine remaining unaffected and the cervical spine being infrequently affected [523]; calcification of the intervertebral ligaments [523]; and hypertrophy of the facet joints of the cervical spine [509].

Radiological features consistent with diffuse idiopathic skeletal hyperostosis (DISH) were found in 15% of patients in a cross-sectional study by Oruk et al [535]; in 20% of patients in a case-control study by Scarpa et al [110]; and in 47% of patients in the thoracic spine in a case-control study by Littlejohn et al [534]. In contrast, no cases of DISH were found among 18 patients with acromegaly in a cross-sectional study by Podgorski, however this may represent a type 2 statistical error due to small number of patients assessed radiologically in this study compared with the previous studies, in which a larger number of subjects were recruited (N=30-54) [509].

Radiological findings from examination of the pelvis in patients with acromegaly include osteophytosis of the parasympheseal area [509], pubic bones (in 71% of female and 53% of male patients) and iliac bones (leading to iliac bone enlargement) [523]; thinning of pubic rami present in two thirds of females [523]; and enthesopathy affecting the iliac crest, the anterior superior iliac spines and the ischial tuberosities [509, 523]. Sacroiliac joint and pubic symphysis widths were found to be normal [509].

The skeleton of the thoracic cavity has been assessed in two cross-sectional studies [509, 523]. Prolongation of the ribs, kyphosis of the thoracic spine and elevation of the sternum leading to a barrel-shaped thorax, was found in 40% of 24 patients with acromegaly in a

cross-sectional study [509]. In the same study, the 'acromegalic rosary' resulting from enlargement of the costochondral junctions on the anterolateral chest walls was observed in 4/24 patients [509]; however clinically palpable costochondral enlargement has been suggested to be a much more common clinical sign in acromegaly than the radiological finding of the 'acromegalic rosary' and was detected in 26/27 patients in an observational study [536].

A summary of the articular changes in patients with acromegaly, in the different anatomical areas, based on radiological assessment can be found in Table 3.2.

Table 3.2. Summary of pathological findings from radiological assessment of different joint sites in patients with acromegaly.

Anatomical site	**Common radiological findings**	**Less common radiological findings**
Lower Limbs		
Hips	Preserved joint space or joint space widening, osteophytes	Joint space narrowing, enthesopathy, cartilage loss, sclerosis of subchondral bone, cyst formation
Knees	Preserved joint space or joint space widening, osteophytes	Joint space narrowing, enthesopathy, cartilage calcifications, capsular ossification, synovitis, thickening of the pre-patellar bursa
Ankles	Preserved joint space, mild osteophytosis	
Feet	Separation of the metatarsal heads	

Upper Limbs		
Hands	Joint space widening, osteophytes	Bone remodelling of metacarpals, squaring of the metacarpal bone heads, tufting of the distal phalanges, enthesopathy of proximal phalanges, calcifications of joint capsules, increased sesamoid index
Shoulders	Joint space widening, osteophytes, cystic changes in the head of humerus, enthesopathy of the greater tuberosity	Joint space narrowing, enthesopathy affecting the clavicle and at the coracoclavicular ligament attachment site
Elbows	Preserved joint space	Degenerative hypertrophic changes, loose bodies
Axial Skeleton		
Cervical spine	Linearization	Hypertrophy of the facet joints, kyphosis, hyperlordosis
Thoracic spine	Kyphosis, osteophytes, increased vertebral dimensions	DISH, posterior scalloping of the vertebral bodies, periosteal appositions
Lumbar spine	Osteophytes, increased vertebral dimensions, increase or reduction of the intervertebral disc space, linearization	DISH, posterior scalloping of the vertebral bodies, scoliosis, hyperlordosis
Pelvis	Osteophytosis of the parasympheseal area, pubic and iliac bones, thinning of pubic rami (in females), enthesopathy	
Thoracic cavity	Prolongation of the ribs, barrel-shaped thorax	'acromegalic' rosary (enlargement of the costochondral junctions on the anterolateral chest wall)

3.3.4. Disease-specific factors associated with arthropathy features in patients with acromegaly

The potential association between putative features of the acromegalic arthropathy (i.e. JSN, osteophytes or progression of the arthropathy) and disease-specific factors (i.e. overall GH exposure, duration of active disease, GH and IGF-1 levels at the time of diagnosis of acromegaly) has been investigated in a number of clinical studies, which have produced contradictory results on some occasions. This is likely related to the small number of participants in most clinical trials, differences in the criteria applied to define or classify arthropathy (clinical vs. radiological vs. combined clinical and radiological criteria) and differences in the biochemical criteria used to define remission of acromegaly (i.e. GH<1.9 mcg/L vs. GH<1 mcg/L in more recent studies).

Hip JSN has been associated with higher disease activity as suggested by the higher pre-treatment GH and IGF-1 standard deviation scores (SDS) in patients with hip JSN compared with patients with preserved joint space width. Additionally, hip JSN has been associated with greater GH exposure (as indicated higher pre-treatment GH level and longer duration of active disease); higher rates of treatment with somatostatin (SMS) analogues; and lower immediate postoperative biochemical disease control rates [111]. Additionally, hip JSN has been associated with patient's age but not with gender, BMI or menopausal status [111]. Deletion of exon 3 of the GH receptor, a polymorphism which enhances GH signaling via its receptor, has been associated with increased prevalence but not severity of hip arthropathy [536].

JSN of the knees has been associated with overall GH exposure, patient's age and female gender, but not with duration of active disease, duration of disease remission, pre-treatment IGF-1 SDS and GH levels, rate of SMS analogue use, patient's age at diagnosis, BMI and menopausal status [111]. A tendency for patients with knee JSN to have lower rates of immediate postoperative disease control was observed, however this did not reach significance after adjustment for age, gender and BMI [111]. Thickening of the femoral

cartilage in the knee joints, as assessed with USS, was not found to be associated with disease activity, including both GH and IGF-1 levels [521].

Regarding hand arthropathy, a linear regression analysis performed in a cohort of patients with controlled acromegaly showed that JSW was associated with higher pre-treatment GH, higher pre-treatment IGF-1 values and younger age at diagnosis. JSW of the hand joints was not found to be associated with the type of acromegaly treatment, the GH and IGF-1 levels at the time the study was performed, or the duration of disease remission [529]. In the same study, men were found to have generally wider joints than women, in both patient and control groups [529]. With regards to other hand measurements (i.e. sesamoid index, tuft width), contradictory results have been reported in different studies. In a case control study by Anton et al, the authors described a gender-related difference in the sesamoid index and tuft width, with higher values in male acromegalic patients [526]. Similar results were reported by Kleinberg et al, however there was a discrepancy in the size of the male and female acromegalic subgroups (15 males vs. 5 females) and the statistical significance of the difference of the sesamoid index in the two genders was not reported [532]. In contrast, Tarhan et al did not find any differences in the sesamoid index, tuft width, joint space measured at the level of the second MCP joint and metacarpal width between male and female patients [525]. In the latter study, all patients had normal GH and IGF-1 levels; however the biochemical disease status was not determined in the study by Anton et al [526].

IGF-1 levels at diagnosis were found to be consistently correlated with the presence of arthropathy (diagnosed radiologically based on the Kellgren and Laurence osteoarthritis criteria) in a variety of different anatomical areas in patients with acromegaly. Patients with pre-treatment IGF-1 SDS in the highest tertile were found to have increased risk of developing OA compared with patients with pre-treatment IGF-1 SDS in the lowest tertile. This was particularly evident in the hips [relative risk (RR) 3.98; 95% confidence interval (CI) 1.69-5.01, $p<0.01$], hand joints (DIP: RR 2.07, 95% CI 1.09-2.21, $p=0.04$; PIP: RR 3.49, 95% CI 1.46-3.57, $p=0.02$; carpometacarpal (CMC): RR 4.76, 95% CI 1.13-5.25, $p=0.04$), knees (RR 2.15, 95% CI 1.14-2.55, $p=0.04$) and the cervical spine (RR 1.29, 95% CI 1.07-

1.31, p=0.03) [492]. Additionally, more diffuse OA appears to be associated with higher pre-treatment IGF-1 levels. Significantly higher IGF-1 SDS at diagnosis were found in patients with OA affecting both hips and knees, more than four intervertebral discs of the cervical spine and more than four DIP joints compared with patients with no OA in these joint areas [492]. Regarding other potential implicating factors, a positive correlation between patients' age at the time of diagnosis and development of OA was found, with patient diagnosed with acromegaly at a younger age showing reduced rates of OA compared with patients diagnosed with acromegaly at an older age, however no significant association was found between OA and pre-treatment GH levels, active disease duration, the different treatment modalities, presence of hypogonadism and hypopituitarism, gender and BMI [492].

In a study by Layton et al. comparing acromegalic patients with and without arthropathy (including both axial and peripheral arthropathy), no difference was found in the two study groups regarding patients' age at diagnosis or GH and IGF-1 concentrations [513]. This is in contrast with the findings from previously mentioned studies, which have shown an association between disease activity markers (i.e. IGF-1 levels at the time of acromegaly diagnosis) and risk of developing arthropathy [492, 529]. This difference is likely related to the small number of patients included in Layton's study (10 acromegalic patients with arthropathy vs. 9 acromegalic patients without) [513] and also to the variation in the criteria used to define arthropathy in the different studies. A combination of clinical and radiological criteria were applied in Layton's study [513], whereas only radiological criteria were used in the two studies by Biermasz [492, 529]. However, patients with arthropathy tended to be older [mean age 46.3 years (range 20-68) vs. 29.2 years (range 18-47), p=0.01] and to have longer duration of active disease [mean disease duration 21.6 years (range 3-50) vs. 7.9 years (range 4-12), p=0.01] compared with patients without arthropathy [513]. In another study, neither the disease duration nor the biochemical markers of acromegaly were found to be correlated with the extent or the severity of peripheral or axial arthropathy [509]. In a non-imaging study, in which the diagnosis of arthropathy was based on patients' self-reported symptoms confirmed by physical examination, presence of arthropathy was positively

associated with patient's age at diagnosis of acromegaly, BMI and female gender. No associations were found between arthropathy and patient's current age, GH and IGF-I levels, pre-treatment IGF-I level and disease control, however more strict biochemical criteria were applied in this study to define disease control (GH <1mcg/L) compared with other studies (target GH <1.9mcg/L for disease remission) [510].

Patient's age at diagnosis of acromegaly appears to be associated with the time of onset of the acromegalic arthropathy. In particular, the time interval between diagnosis of acromegaly and onset of symptoms of arthropathy was significantly shorter in patients diagnosed with acromegaly over the age of 40 years compared with patients with onset of acromegaly at the ages 31-40 years or below the age of 30 (4.1 vs. 10.7 years and 9.7 years respectively, $p<0.001$) [530]. In the same study, patients with severe symptoms, defined as severe joint pain necessitating medical treatment or leading to disability requiring surgical assessment with or without surgical intervention, were noted to have two to three times higher GH levels at the time of cranial radiotherapy, which for the majority of patients was the first line treatment for their acromegaly in this cohort, compared with patient with mild arthropathy or no joint symptoms [530]. The percentage of GH reduction following radiotherapy was not different between patients whose symptoms of arthropathy improved or worsened following treatment [530].

Radiological features consistent with DISH, were more commonly seen in patients of older age (69% of patients older than 50 years had DISH, compared with 29% of patients below the age of 50 years, $p<0.001$) and in patients with longer duration of acromegaly (11.5 years vs. 5.4 years, $p<0.05$) [534]. No difference was noted in the age of onset of acromegaly and the GH levels among patients with and without DISH [534]. Regarding axial arthropathy in acromegaly, disease duration was positively correlated with vertebral body height and height of intervertebral space [110].

An ultrasonographic study of cartilage thickness, showed positive correlation between patients' age and disease duration with the cartilage thickness of the wrist and knee in patients with active acromegaly. No correlation was found between GH/IGF-1 values and joint space

width. For healthy controls, age was positively correlated with cartilage thickness at the wrist only [520]. In the longitudinal arm of the same study, in which patients with active acromegaly received treatment with octreotide-LAR for 12 months, the percentage of reduction in the GH values was found to correlate with the reduction in the wrist cartilage thickness, while the percentage of the IGF-1 decline correlated with the reduction in knee cartilage thickness [520]. A summary of the associations described above is provided in Table 3.3.

Overall, taking all the above data into account, it can be said that among the various acromegaly-specific factors, pre-treatment IGF-1 levels and GH exposure (calculated based on the GH levels and the duration of active disease) have been more consistently reported to be related with certain aspects of the acromegalic arthropathy and particularly, radiological OA in most anatomical areas [492], JSN in the hips and knees [111], joint space widening of the hands [521] and the presence of more diffuse and severe arthropathy [492]. On the contrary, duration of disease remission, current GH and IGF-1 levels have not been shown to be associated either with clinical or radiological arthropathy, possibly suggesting that the joint alterations that occur during the active phase of the disease are permanent and do not reverse with successful treatment of the disease and sustained biochemical remission [437, 521, 529].

Table 3.3. Summary of associations between acromegaly-specific factors with different aspects of arthropathy in patients with acromegaly. The "+" symbol suggests that there is a positive correlation between the acromegaly-specific factor and the arthropathy feature. In contrast, the "-" symbol suggest a negative correlation between the two variables. When studies have shown no evidence of an association between two parameters, this has been recorded as "nil" on the table. The numbers in brackets provide the literature reference in which the presence or absence of the correlation was described.

Arthropathy Feature	**FACTOR**														
	Pre-treatment IGF-I	Pre-treatment GH	Age at diagnosis	Active disease duration	GH exposure	Duration of disease remission	Current IGF-I	Current GH	Current age	Female gender	Male gender	BMI	SMS treatment	Treatment modalities	d3GHR
Clinical OA	Nil [540]		+ve [540]				Nil [540]	Nil [540]	Nil [540]	+ve [540]		+ve [540]			
Radiological OA	+ve [514]	Nil [514]	+ve [514]	Nil [514]						Nil [514]		Nil [514]		Nil [514]	
Clinical & Radiological OA	Nil [440]	Nil [440]	Nil [440]	+ve [440]					+ve [440]						
OA severity	+ve [514]	+ve [506]													
Bilateral / Diffuse OA	+ve [514]														
Hip OA															+ve [527]
Hip JSN	+ve [501]	+ve [501]			+ve [501]				+ve [501]	Nil [501]	Nil [501]	Nil [501]	+ve [501]		
Knee JSN	Nil [501]	Nil [501]	Nil [501]	Nil [501]	+ve [501]	Nil [501]			+ve [501]	+ve [501]		Nil [501]	Nil [501]		
Femoral cartilage thickness	Nil [511]	Nil [511]	+ve [511]	+ve [511]			Nil [512]	Nil [512]	Nil [511]						
Hand JSW	+ve [515]	+ve [515]	-ve [515]			Nil [515]	Nil [515]	Nil [515]			+ve [515]			Nil [515]	
Wrist cartilage thickness	Nil [511]	Nil [511]	+ve [511]	+ve [511]					Nil [511]						
Sesamoid Index & Tuft width											+ve [497, 518], Nil [516]				
Vertebral body height				+ve [523]											
Spinal DISH		Nil [524]	Nil [524]	+ve [524]					+ve [524]						

3.3.5. Natural history of acromegalic arthropathy

There is only one prospective cohort study, investigating the radiological progression of the arthropathy in acromegalic patients with long-term disease control [537]. Fifty-eight patients, with mean duration of disease remission of 17.6 years were studied over a mean period of 2.6 years. The Osteoarthritis Research Society International (OARSI) classification was applied to define progression of joint disease (i.e. 1-point increase in the JSN or osteophyte score). Progression of JSN was noted in 74% of patients and was more prominent in the hand joints, while progression of osteophytes was observed in 72% of patients, mainly in the knees [537]. Regarding factors associated with progression of arthropathy, JSN progression in the hips and knees was positively associated with baseline IGF-1 SDS and the use of somatostatin analogues, while age was a risk factor for JSN progression in the hips and hands. Osteophyte progression in the knees was associated with the d3-growth hormone receptor (d3-GHR) polymorphism, patient's age, somatostatin analogue treatment; and with duration of active disease in the hands [537].

Clinical progression of the acromegalic arthropathy, based on patients' symptomatology, was evaluated in a prospective study by Claessen et al [516]. Worsening function scores of the hands, as assessed by the Australian/Canadian Osteoarthritis Hand Index (AUSCAN), and worsening stiffness and function scores of the lower limbs, as assessed by the Western Ontario McMaster Universities Osteoarthritis Index (WOMAC), were found in 58 patients with long-term remission of acromegaly over a mean follow-up period of 2.6 years [516]. No difference in the AUSCAN and WOMAC pain scales was noticed between baseline and follow-up assessment. Joint pain and functional impairment at baseline were identified as risk factors for clinical progression of the arthropathy in both hands and lower limbs, using a binary regression analysis model. The change in AUSCAN and WOMAC pain, stiffness and function scores at baseline and follow-up was not different between patients with and without evidence of radiological progression of acromegalic arthropathy and therefore no association was identified between symptomatic worsening and radiological progression of JSN and osteophytes [516].

Progression of clinical arthropathy over time, based on symptoms alone, in patients who have previously received treatment for acromegaly, was also reported in a retrospective longitudinal study by Dons et al [530]. Worsening of self-reported pain was found in 61.9% of patients, while 40.4% patients developed severe clinical arthropathy, based on a non-standardized 5-scale grading system of joint pain and degree of disability [530]. Joint surgery was required in 10.6% of patients in the same cohort. Progression of JSN on radiographic examination occurred predominantly in patients with severe clinical arthropathy and was observed in 76% and 78% of weight bearing and non-weight bearing joints respectively in these patients. For patients with mild/moderate severity of arthropathy the percentage of JSN progression was 30% and 22% for weight bearing and non-weight bearing joints respectively [530].

Overall, the acromegalic arthropathy appears to progress both in radiological and clinical terms, even after achieving disease remission, which suggests that the articular changes that occur under the effect of longstanding GH and IGF-1 excess are irreversible and predispose to ongoing intra-articular damage and progression in the severity of arthropathy.

3.3.6. The effect of treatment for acromegaly on the acromegalic arthropathy

Reduction in the cartilage thickness in both non-weight and weight-bearing joints has been reported in treatment-naïve patients with active acromegaly, following treatment with somatostatin analogues. Results from three cohort studies, in which ultrasonographic measurements of cartilage thickness in the right shoulder, right wrist, both knees and both heel tendons were performed, have shown significant reduction in the joint cartilage thickness after 6 months treatment with Octreotide LAR, 0.3-0.6mg daily [519], 12 months treatment with Octreotide LAR, 10-40mg every month [520] and 12 months treatment with Lanreotide, 30mg every 10-14 days [518]. The reduction observed in non-weight bearing joints (shoulder, wrist) was greater than the reduction seen in the knees and heel tendons [518-520]. The degree of reduction in the cartilage thickness was correlated with the duration of treatment. Following three months of treatment with Octreotide LAR or Lanreotide a significant

reduction in the cartilage thickness was noticed only in the right shoulder and right knee respectively, however following 12 months of medical therapy significant reduction was noticed in all joint areas [518, 519]. Patients who achieved biochemical disease control following 12 months of Lanreotide demonstrated greater reduction in the cartilage thickness compared with patients who still had active disease at the end of the 12-month period of medical treatment [518]. There was a positive correlation between the percentage of decrease in GH level and the reduction in wrist thickness, and between the percentage of decrease in IGF-1 level and the reduction in right and left knee thickness [520]. No difference in the degree of cartilage thickness reduction was noticed between patients with duration of active acromegaly greater or less than 10 years [518-520].

The effect of the treatment for acromegaly on the severity of acromegalic arthropathy was also assessed by Layton et al, in a heterogeneous cohort of 9 patients with active disease; 4 patients were treatment naïve and 5 patients had received previous treatment with a combination of pituitary surgery, radiotherapy and/or medical treatment with bromocriptine. All 9 patients had clinical evidence of arthropathy at baseline and the severity of joint disease was reassessed after 1-21 months, following additional treatment which consisted of SMS analogue therapy in 6 patients, trans-sphenoidal surgery in 1 case, combination therapy with pituitary surgery and SMS analogue, or SMS analogue therapy plus bromocriptine in the remaining 2 cases. Based on a non-validated grading system for the evaluation of the severity of arthropathy, consisting of a combination of clinical and radiological criteria, treatment of active acromegaly in these 9 cases led to an improvement of clinical arthropathy (reduced pain score, crepitus and increased functional ability), although no change in the structural features of the joints was reported. However, results were presented in a descriptive way and no statistical analysis of the data was performed [513].

In a prospective study of 58 patients with acromegaly, following multi-modality treatment, patients treated with SMS analogues were found to have increased risk [expressed by the use of odds ratios (OD), adjusted for age, gender, BMI and IGF-1 SDS] of developing JSN progression in the knee (adjusted OR=3.5, 95% CI 1.12-10.28; p=0.02) and hip (adjusted

OR=4.3, 95% CI 1.03-17.80; p=0.045) joints and osteophyte progression in the hands (adjusted OR=4.2, 95% CI 1.37-12.85; p=0.01) compared with patients treated with surgery. Additionally, a positive correlation was found between duration of treatment with SMS analogues and severity of arthropathy [537]. A possible explanation for the above findings is that these patients may have had a higher overall exposure to abnormally elevated GH/IGF-1 levels and more difficult to control acromegaly. In fact, with the exception of two cases, SMS analogues were used as an adjuvant treatment to previous pituitary surgery and/or radiotherapy, and patients who were on medical therapy had longer duration of active disease and higher IGF-1 SDS, suggestive of a less favorable biochemical disease profile in these patients.

A number of studies have previously reported on the effects of somatostatin analogues on arthralgia, but not on structural articular changes. When assessing the clinical efficacy of short-acting subcutaneous octreotide [538], long-acting intramuscular Octreotide-LAR [539] and slow-release intramuscular lanreotide [540], studies have reported significant improvement to arthralgia (amongst other symptoms of active acromegaly i.e. headaches, soft tissue swelling, hyperhidrosis) following the use of somatostatin analogues over a period of several months. These studies were performed on groups of patients with active acromegaly, who were either treatment naïve prior to the start of SMS analogue therapy, or had previous pituitary surgery with or without radiotherapy but failed to achieve remission. Arthralgia was assessed using a 4-point 0-3 scale, with 0 indicating absence of arthralgia and 3 indicating severe arthralgia. More recently, results based on the PRIMARYS study data, in which 90 treatment naïve patients with acromegaly were treated with 4-weekly Lanreotide Autogel intramuscular injections 120mg, showed improvement in arthralgia (evaluated using the Patient-assessed Acromegaly Symptom Questionnaire or PASQ), which was sustained over a treatment period of 48 weeks [464].

3.3.7. Acromegalic arthropathy and patients' symptoms

Articular pain has been the most commonly reported symptom with a prevalence ranging between 56-94% in different studies [107, 109, 112, 437, 509, 510, 517, 530], while crepitus is the commonest clinical sign [509, 512]. In a large retrospective study of 229 patients with acromegaly, 36 patients (15.7%) had joint symptoms severe enough to require further investigation with joint imaging. Bilateral joint involvement was noted in the majority of cases, particularly when arthropathy affected the shoulders, hips or knees [511]. A further 62% of the patients had mild symptoms that were not considered clinically significant to be investigated further. Only a minority of patients with acromegalic arthropathy require surgical repair. Hip replacement was found to be less prevalent amongst patients with acromegaly compared with primary OA patients [108]. Joint replacement surgery in acromegalic patients is generally required for weight-bearing joints (hips and knees) and prevalence rates range between 3.4%-10.6% in different studies [107, 511, 516]. Joint swelling, particularly in the knee and hand joints, has also been reported, however less commonly than articular pain [109, 512, 517].

There is variation in the prevalence of joint symptoms in the different anatomical areas, with articular pain and stiffness being more commonly reported in the hands and spine, followed by the knee and hip joints [109, 112]. When compared with age and gender matched controls, patients with acromegaly in long-term remission had significantly higher pain/stiffness scores in all anatomical areas (hip, knee, hand, cervical and lumbar spine), using a standardized questionnaire to capture participants' symptoms and signs of arthropathy [109]. Assessing pain, stiffness and physical impairment of the lower limps with the validated Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), significantly higher scores were found in the patient group compared with controls, indicating a greater degree of disability of the hip and knee joints [109]. When compared with patients with primary osteoarthritis, patients with treated acromegaly and long-term remission demonstrated better outcome scores on the WOMAC scale, probably the result of the significantly lower prevalence of JSN in the acromegalic group [108]. In fact, JSN was found to be correlated positively with the

WOMAC score after adjusting for age, gender and BMI [108]. Additionally, self-reported knee pain was less prevalent in acromegaly compared with OA patients; however there was no difference in the hip pain prevalence [108].

Symptoms of axial arthropathy are present in up to 72% of patients (significantly more prevalent than in controls) and affect primarily the lumbosacral, followed by the cervical spine [110]. Significantly reduced spinal motility and thoracic cage expansion has been found in patients with acromegaly compared with controls in a case-control study [110], however normal or even increased spinal motility has also been documented in other studies [509, 512].

Joint-related symptoms influence significantly patients' perceived quality of life (QoL), based on the results from a number of validated generic and disease-specific questionnaires. Acromegalic patients with joint symptoms were found to have significantly lower scores in all QoL questionnaires compared with patients without joint symptoms. This was primarily related to the negative influence of arthropathy on the domain of physical activities and functioning and to a lesser extent due to its impact on the psychosocial subscales of the questionnaires [107, 112]. Radiological arthropathy was not associated with impaired QoL, however, as no difference in QoL scores was noticed among patients with and without articular abnormalities on radiological assessment [112].

3.3.8. Acromegalic Arthropathy vs. Primary Osteoarthritis

The term "osteoarthritis" has often been used in the literature to describe the arthropathy in acromegaly. It appears that the acromegalic arthropathy shares common features with primary OA, particularly in the more advanced stages of the acromegalic arthropathy, when JSN develops. Additionally, both types of arthropathy are degenerative and non-inflammatory. However, joint space widening (the commonest radiological finding in patients with acromegaly) is a unique feature of the acromegalic arthropathy and is not encountered in patients with primary OA. Therefore classification systems of OA severity, such as the Kellgren-Lawrence grading system, are not applicable for acromegalic patients, as they are

based on the presence of JSN only and do not account for JSW [541]. The preserved or widened joint space particularly in the initial stage of the acromegalic arthropathy, can possibly explain the better patient reported outcomes in acromegaly compared with primary OA patients [108]. Osteophytosis is a common feature in both acromegalic arthropathy and OA; however its prevalence has been found to be higher in patients with acromegaly [108].

In a recent case control study, MRI was used to perform structural evaluation of knee joints in 26 patients with acromegaly and 25 controls with primary OA. A lower prevalence of bone cysts and bone marrow lesions, but higher prevalence of severe cartilage defects and osteophytosis was found in the acromegalic group compared with patients with OA. Additionally, patients with acromegaly had increased cartilage thickness and higher cartilage T2 relaxation times, with the authors concluding that this was indicative of altered biochemical cartilage composition in the acromegalic group [522].

Deletion of exon 3 of the GH receptor (d3-GHR), a polymorphism which enhances the transduction of GH signaling, was found to be associated with increased prevalence of complications of acromegaly including arthropathy, predominantly in the hip joints [263]. Similarly, the d3-GHR was found to be associated with symptomatic osteoarthritis in non-acromegalic females, particularly in the hip joint [542], suggesting that the acromegalic arthropathy may be sharing some common pathophysiological pathways with primary OA.

Overall, it remains unclear whether the arthropathy that patients with acromegaly develop is a distinctive type of arthropathy or whether it represents premature OA. The preserved or widened joint space, due to articular cartilage thickening as a result of chronic exposure to GH and IGF-1 excess is certainly unique to patients with acromegaly, however as a result of a continuous degenerative process, a phenotype similar to OA develops in later stages, with the presence of JSN and osteophytes. Further studies using modern imaging modalities (i.e. MRI scan) are required to characterize further the arthropathy in patients with acromegaly and identify any possible differences with OA. Table 3.4 summarizes the similarities and differences between acromegalic arthropathy and OA.

Table 3.4. Comparison between acromegalic arthropathy and primary osteoarthritis. Differences and similarities.

	Acromegalic Arthropathy	**Primary Osteoarthritis (OA)**
Joint space widening	Very common	Absent
Joint space narrowing	Rare	Very common
Osteophytes	Very common	Common
Cartilage defects	Very common	Very common
Bone cysts	Rare	Common
Bone marrow lesions	Rare	Very Common

3.4. CONCLUSIONS

Based on this extensive literature review, the acromegalic arthropathy is a degenerative polyarthropathy, which can affect any joint of the appendicular or axial skeleton. Preserved or widened joint space with osteophytes is the predominant radiological feature observed in the joints of the appendicular skeleton. Joint space narrowing is found in a minority of patients with acromegaly and is a feature of a more advanced joint disease due to prolonged intra-articular trauma and cartilage damage. In the axial skeleton, increased vertebral dimensions and osteophytosis are the commonest findings. Articular changes in acromegaly do not only refer to alterations in the skeleton, but also involve periarticular structures and other compartments of the joints. Changes in the articular cartilage thickness, synovitis with presence of effusion, enthesopathy (mineralisation at the point of a ligament attachment to the bone) and capsular calcifications have also been reported.

Arthropathy is the commonest complication of acromegaly and the main cause of morbidity in these patients, which significantly accounts for the impaired quality of life observed even long after successful treatment of acromegaly and biochemical remission. Progression of arthropathy, both in radiological and clinical terms, has been observed in patients with long-

term disease control, which suggests that articular changes develop early in the course of acromegaly and do not reverse even following successful treatment. Further studies are required to investigate whether early diagnosis and aggressive management of acromegaly can help preventing the development of arthropathy or reverse any early articular changes. Additionally, the role of the GH receptor antagonist, pegvisomant, on altering the effects of GH on the skeleton has not been examined.

Undoubtedly, early detection of acromegaly and prevention of any disease complications would be the ultimate target in the management of the disease. However, the insidious onset of acromegaly and its slow and gradual progression, often leads to delayed diagnosis, which occurs when patients have already developed complications from the long-standing disease activity. Enquiring about joint-related symptoms should form part of the routine follow-up of patients with acromegaly. Therapeutic options at present include either conservative management with symptomatic pain relief and possibly physiotherapy or in more advanced cases referral to orthopaedic services for consideration of joint replacement surgery; however no strategies are currently available to reverse or prevent the arthropathy in patients with acromegaly.

A limitation of this literature review is related to the heterogeneity in the methodology of the original studies included in the review. Different imaging modalities and different diagnostic criteria have been applied to define arthropathy in patients with acromegaly and assess joint-related symptoms in the available original studies, across different chronological eras. Additionally, the results of many of the studies included in this review are based on observations from small cohorts of patients with acromegaly, which is limiting the quality of these studies. Well-designed observational studies, on large cohorts of patients with acromegaly, using modern imaging modalities (i.e. MRI scan) of the joints are required to increase further the understanding on the arthropathy, which remains one of the unresolved issues in patients with acromegaly.

CHAPTER FOUR

ASSESSMENT OF KNEE BONE SHAPE IN PATIENTS WITH ACROMEGALY

4.1. ABSTRACT

Objective: Arthropathy is the commonest morbidity in patients with acromegaly and one of the main factors impacting on patients' quality of life even after successful treatment of acromegaly and long-term disease control. To this date there are no specific therapeutic strategies available and options include symptomatic control with analgesics and joint replacement surgery in more advanced cases. The majority of the current knowledge on the acromegalic arthropathy is derived from studies using conventional x-rays. This study aims to characterize the features of the acromegalic arthropathy using modern imaging techniques.

Design: This is a two-arm study. In the cross-sectional arm, 60 patients with acromegaly (29 males, mean age 54.8±12.9yrs) at different stages of disease activity were compared with 300 age/gender-matched controls from the publicly available Osteoarthritis Initiative (OAI) database via propensity score matching. An additional control group of 886 individuals without any evidence of knee osteoarthritis on serial MRI scans, also referred as non-OA group, was selected from the OAI. In the longitudinal arm, bone shape data from 42 patients with acromegaly were compared at baseline and after at least 12 months of follow-up.

Methods: Acromegaly patients attended for bilateral knee MRI scan at baseline and after at least 12 months of follow-up. Knee bone shape, joint space width (JSW) and cartilage thickness were measured based on automated segmentation of MR images of knee bones and calculation of bone area using active appearance models.

Results: Acromegaly patients had increased medial JSW compared with controls [6.21mm (95% CI 6.03-6.40) vs. 5.78mm (95% CI 5.70-5.87) respectively, $p<0.001$] and increased lateral and medial femorotibial cartilage thickness. Patella and medial tibia bone areas were also increased in acromegaly patients. B-score (a biomarker associated with severity and risk

of progression of OA) was significantly higher in patients compared with controls [1.7 (95% CI 1.32-2.08) vs. 1.01 (0.84-1.18) respectively, p=0.001].

Thirty-five percent (n=21) of acromegaly patients had B-score ≥2, which is indicative of OA. These patient had higher GH levels at the time of diagnosis of acromegaly and required a higher number of therapeutic interventions during the disease management compared with patients with B-score <2 (n=39). Additionally, patients with B-score ≥2 had significantly larger femoral, tibial and patella bone areas, increased medial JSW and lateral and medial femorotibial cartilage thickness compared with the remaining patients.

However, when patients with B-score <2 (within the B-score reference range) were compared with the non-OA control group, acromegaly patients still demonstrated differences in bone shape, with increased bone area at the lateral patella [patients: 695mm^2 (95% CI 675-716), non-OA controls: 670mm^2 (666-674), p=0.017], medial patella [patients: 547mm^2 (95% CI 531-563), non-OA controls: 523mm^2 (95% CI 520-526), p=0.004] and medial tibia [patients: 1166mm^2 (95% CI 1141-1191), non-OA controls: 1137mm^2 (95% CI 1132-1142), p=0.02]; increased B-score [patients:0.69 (95% CI 0.36-1.02), non-OA controls: 0.018 (95% CI -0.05 – 0.08), p<0.001]; and increased lateral and medial femorotibial cartilage thickness. Comparison between baseline and follow-up scans for the 42 patients who completed the longitudinal arm of the study did not show any change in the bone size area, B-score or JSW.

Conclusions: Acromegaly patients despite higher B-score and larger bone area particularly of the patella and medial tibia bones have preserved and/or increased joint space due to increased cartilage thickness, which distinguishes acromegalic arthropathy from osteoarthritis. The higher pre-treatment GH values and the higher number of therapeutic interventions seen in patients with B-score ≥2, indicate that overall exposure of peripheral tissues to excessive GH levels is a risk factor for more pronounced changes to the knee bone shape and potentially more severe arthropathy.

4.2. INTRODUCTION

The structural pathology of the arthropathy in patients with acromegaly has mostly been evaluated using X-rays. However, radiological evaluation is semiquantitative and is based on two-dimensional imaging and has often been considered inadequate for the assessment of joint alterations in patients with osteoarthritis. This is due to its low sensitivity to detect structural changes of early OA, its inability to visualize certain pathologies frequently encountered in patients with OA (i.e. bone marrow lesions, periarticular cystic lesions, effusions, meniscal tears) and also due to the low detection rate of longitudinal structural changes [543].

MRI allows for detailed detection of joint structural alterations based on three-dimensional imaging, providing quantitative data for the assessment of bone and cartilage [544]. MRI has been increasingly used in OA research for the assessment of structural pathologies, however in acromegaly data on arthropathy are predominantly derived from studies using conventional radiography, as described in Chapter 3 of this Thesis. Image analysis has progressed significantly and active appearance models (AAMs), a form of statistical shape modeling (SSM) can be combined with MRI and allow automatic segmentation of all bone surfaces from MR images in order to create a model, which can be used to quantify bone shape and structural pathologies of the joints [545].

Subchondral bone area has been identifying as having an important role in the development and progression of osteoarthritis. Osteophytosis and joint space narrowing have been associated with increase in the subchondral bone area and women with radiographic evidence of knee OA were found to have larger tibial subchondral bone areas compared with non-OA women [546]. Additionally, tibial subchondral bone area has been associated with presence and severity of knee cartilage defects and tibial subchondral bone expansion has been related with progression in tibiofemoral cartilage defects over time and reduction in the cartilage volume [547, 548], indicating that changes in the subchondral bone shape is a clinical sign of early OA, which precedes cartilage loss.

Differences in femoral and tibial bone shape have been identified between patients with and without OA, including widening and flattening of the femoral and tibial condyles, an increased ridge of osteophytic growth around the cartilage plate of femur and tibia and increased size of the patellar cartilage plate and osteophytic ridge in OA-patients [549]. Bone shape can be predictive of subsequent development of radiographic OA in knees without radiographic evidence of OA at baseline and therefore can be used as a potential biomarker to identify patients at risk of developing OA at a later stage [549]. In addition to this association between bone shape and structural OA progression, bone shape has also been associated with total knee replacement (TKR). In a case-control study, patients who had previously undergone TKR had significantly increased bone shape, with the femoral bone shape exhibiting the strongest association with TKR [550].

More recently, based on bone shape measurements by applying statistical shape modeling on knee MR images, a new marker, namely B-score, was proposed as a single standard quantitative measure of OA status [551]. The B-score refers to the femoral bone shape, which in previous studies has shown the strongest association with severity and structural progression of knee OA. The B-score showed superior ability to discriminate among the different degrees of severity of OA over traditional stratification systems, such as the Kellgren-Lawrence grade (KLG), which is based on conventional radiological assessment of joints. Finally higher B-scores were associated with a variety of relevant clinical OA outcomes, including risk of current and future joint pain, functional limitations and risk for total knee replacement [551].

As discussed previously in Chapter 3 of this Thesis, arthropathy is the most common morbidity in patients with acromegaly and joint symptoms associated with this is one of the unresolved issues for these patients, even despite successful treatment and biochemical disease control. Current knowledge about the arthropathy in acromegaly is predominantly based on conventional radiological joint assessment, with very limited input from MR imaging. The use of this novel method of bone shape measurement has significantly advanced the research in the OA field in the recent years, however it has never been applied in patients

with acromegaly. The arthropathy associated with acromegaly shares at least in part some common features with OA (i.e. osteophytosis, cartilage defects); however it demonstrates some distinctive features mainly in the form of preserved joint space. In this chapter, bone shape is explored for the first time in patients with acromegaly, based on the results of a case-control study.

4.3. PATIENTS AND METHODS

4.3.1. Study Design

The study consists of two arms: a cross-sectional to compare bone shape between patients with acromegaly and control individuals matched for age and gender; and a longitudinal arm to assess for changes in the bone shape in patients with acromegaly during a follow-up period of 12 months.

The cross-sectional arm of the study aims to describe bone shape in a cohort of patients with acromegaly and characterize the acromegaly-related joint alterations, using bone shape based on knee MRI as a surrogate marker, and compare these finding against controls. The longitudinal arm of the study aims to describe the natural history and progression of the arthropathy in acromegaly and determine whether any change in bone shape occurs over time progression, taking into account the disease activity status.

4.3.2. Study Outcomes

The primary outcome of this study was to establish a MRI cohort of patients with acromegaly and collect cumulative clinical and imaging data to determine bone shape in acromegaly, based on 3D MRI of the knee joint and compare this with control individuals matched for age and gender.

Secondary outcomes of the study include:

- Divide the acromegaly cohort into two subgroups based on the evidence of osteoarthritis on MRI and compare the two groups for differences in bone shape as well as differences in acromegaly-related parameters (i.e. biochemical markers of disease activity, treatment history, duration of active disease and disease remission), in order to identify factors specific to acromegaly which may be related to the presence and severity of arthropathy in these patients.
- Identify acromegaly patients without knee OA on MRI and compare bone shape with a selective control group without evidence of knee OA based on serial MRI scans over a 5-year follow-up period (“non-OA” group).

- Correlate severity of knee OA on MRI with patient-reported outcomes and markers of quality of life, comparing patient reported outcomes between acromegaly patients with and without OA.
- Evaluate whether there is progression of the acromegaly-related arthropathy with time by comparing bone shape in patients at baseline and after a follow-up period of 12 months.

4.3.3. Participants' recruitment

Sixty-two consecutive patients with acromegaly were recruited in the cross-sectional arm of the study. Patients were approached when attending for their routine appointments in the Endocrine clinics at Leeds Teaching Hospitals NHS Trust. Medical records of patients who expressed an interest to participate to the study were screened for the inclusion/exclusion criteria. Both patients with active disease and disease remission were approached, as well as patients with newly diagnosed acromegaly and patients under long-term clinic follow-up after treatment. Informed consent was obtained from all participants. The study was approved by the South East Scotland Research Ethics Committee (Reference ID: 14/SS/1059).

The control group was selected from participants of the Osteoarthritis Initiative (OAI) database using propensity score matching. Each patient with acromegaly was matched with 5 controls of similar age and the same gender. The OAI study is a multi-centre, longitudinal, observational study aiming to determine the natural history of knee osteoarthritis (OA) and risk factors for the progression of this. Bilateral knee MR images and clinical data were collected prospectively from 4796 individuals with clinical evidence of knee OA at risk of disease progression and also participants without symptoms of OA who are at risk of developing OA (http://oai.epi-ucsf.org/datarelease/docs/StudyDesignProtocol.pdf). The control group for the current study was derived from the latter subgroup of the OAI. The data from the OAI are publicly available (https://data-archive.nimh.nih.gov/oai/). All participants provided informed consent to the OAI.

In addition to the above control group, a total of 886 individuals, who did not have any evidence of knee OA based on serial MRI scans over a 5-year follow-up period, were identified from the OAI database. These are referred as the "non-OA" group and were compared with acromegaly patients with a B-score of <2, which is considered to indicate absence of arthropathy (as explained later in the methodology section), based on quantitative MRI assessment. The individuals of the non-OA group were not matched for age or gender with patients.

4.3.4. Inclusion/Exclusion criteria

Inclusion criteria to the study included:

- Patients diagnosed with acromegaly at different stages of their disease course (i.e. newly diagnosed patients, as well as patients under surveillance following previous treatment) and at different degrees of disease activity (i.e. active disease, disease remission).
- Age ≥ 18 years old.
- Able to provide informed consent.

Exclusion criteria included:

- Patients with prior diagnosis of primary inflammatory arthritis, including rheumatoid arthritis, gout, polymyalgia or connective tissue disease that can potentially affect the knee joints.
- Age <18 years old.
- Patients with contraindications to MR imaging:
    - Patients with pacemaker not compatible with MRI
    - Patients with surgical clips within the head
    - Patients with certain inner ear implants
    - Patients with neuro-electrical stimulators
    - Patients with metal fragments within the eye or head

- Patients with previous bilateral knee replacements.
- Patients unable to give informed consent

4.3.5. Study Visits and Data Collection

Eligible patients with acromegaly recruited to the study were invited to attend for two study visits within a space of at least 12 months. Each visit took place at the Leeds Institute of Rheumatic and Musculoskeletal Medicine at Chapel Allerton Hospital, Leeds, where bilateral knee MRI scans and patients' interviews for clinical data collection were combined in a single visit. The MRI acquisition process is described in more detail in another section of the methodology.

During the first study visit a detailed past medical history was taken, with a special focus on information about acromegaly-related history (i.e. year of onset of symptoms of acromegaly; year acromegaly was diagnosed; treatments that the patient had undergone at the time of the study; duration of active disease – defined as the time from onset of symptoms of acromegaly until biochemical disease control was achieved; duration of disease remission – defined based on GH <1mcg/L and IGF-1 within the age-specific reference range; presence of hypopituitarism as a results of the pituitary tumour or treatment thereof; and presence of other acromegaly-related comorbidities) and medical history related to arthropathy (i.e. presence of knee pain – current or previous; knee pain score using a 0-10 numerical scale, with 0 indicating absence of pain and 10 indicating the most severe pain; duration of knee patient; presence of pain at any other joint site; history of previous joint surgery and whether this was related to arthritis or trauma/fracture; use of analgesia or other local therapies for arthralgia). Biochemical data (including levels of GH, IGF-1 and results of the remaining anterior pituitary hormone profile) were collected to determine the disease activity status of the patients at the time of the study. All patients had recently attended the Endocrine outpatient clinic (through which they were initially approached to the take part to the study) and therefore their biochemical investigations were up-to-date at the time of the first study visit (all blood tests were undertaken within two months of the initial study visit). Measurements

of height and weight and calculation of patient's BMI was also performed. All patient's clinical data were collected using the clinician's case report form, a copy of which can be found in Appendix II of this thesis.

During the second study visit, following a period of at least 12 months, acromegaly-related history was reviewed for additional treatments that patients may have received and any changes in the disease activity since the previous study visit, by collecting further biochemical data, including up-to-date GH and IGF-1 levels. Additionally, patients were interviewed further regarding arthropathy-related symptoms, similar to the baseline visit. Anthropometric measurements (height and weight) were also repeated.

4.3.6. MR image acquisition and quantitative measures

Patients with acromegaly underwent bilateral non-contrast knee MRI scan, using a Siemens Trio 3.0 Tesla scanner at baseline and at one year follow-up. High-resolution knee MRI images were acquired to determine 3-D bone shape. MRI scans for the OAI participants were already available. The protocol for image acquisition for the OAI participants has been previously published in detail [552]. The following MR image sequences were used for analysis:

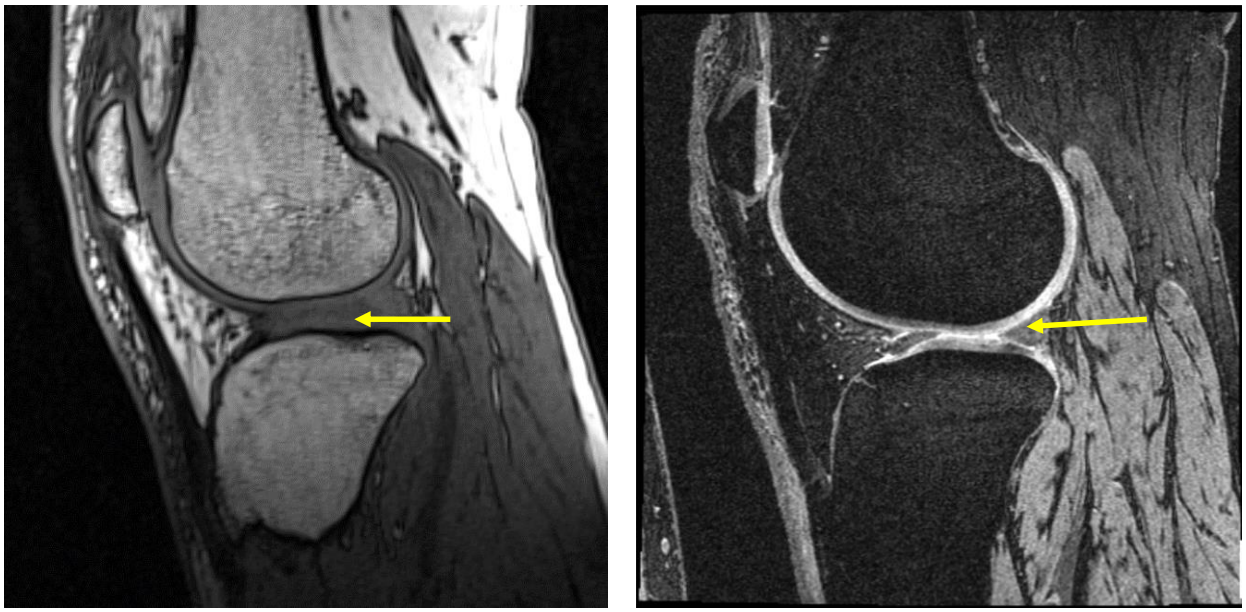
i. Vibe sequence: 3D T1 sagittal VIBE, voxel size (x/y/z) was 0.6/0.6/0.6mm

ii. TSE sequence: 2D Proton Density TSE sagittal with fat saturation, 0.4x0.4x3.6

4.3.6.i. Bone Measurements / Statistical shape modeling (SSM)

Quantitative 3D bone shape data were provided by Imorphics (Manchester, UK). The vibe sequence was of high resolution, but retained fat signal, making the bone white. Most Imorphics models/publications used fat saturation, in which bone appears black (Figure 4.1). Therefore, it was necessary to produce models, which were capable of automatically segmenting bone surfaces (Figure 4.2).

Figure 4.1. (i) Acromegaly vibe image, lateral condyle, sagittal. Bone edges are well visualized, however cartilage edges and meniscus are not well visualized in the tibiofemoral joint (arrow). (ii) Dual-echo at steady-state water-excitation (DESS-we) fat saturated image, lateral condyle, sagittal. Most of previously published work from Imorphics has used this sequence of MR images, with fat signal remover. Cartilage edges and meniscus are well visualized in the tibiofemoral joint (arrow), allowing for accurate measurement.

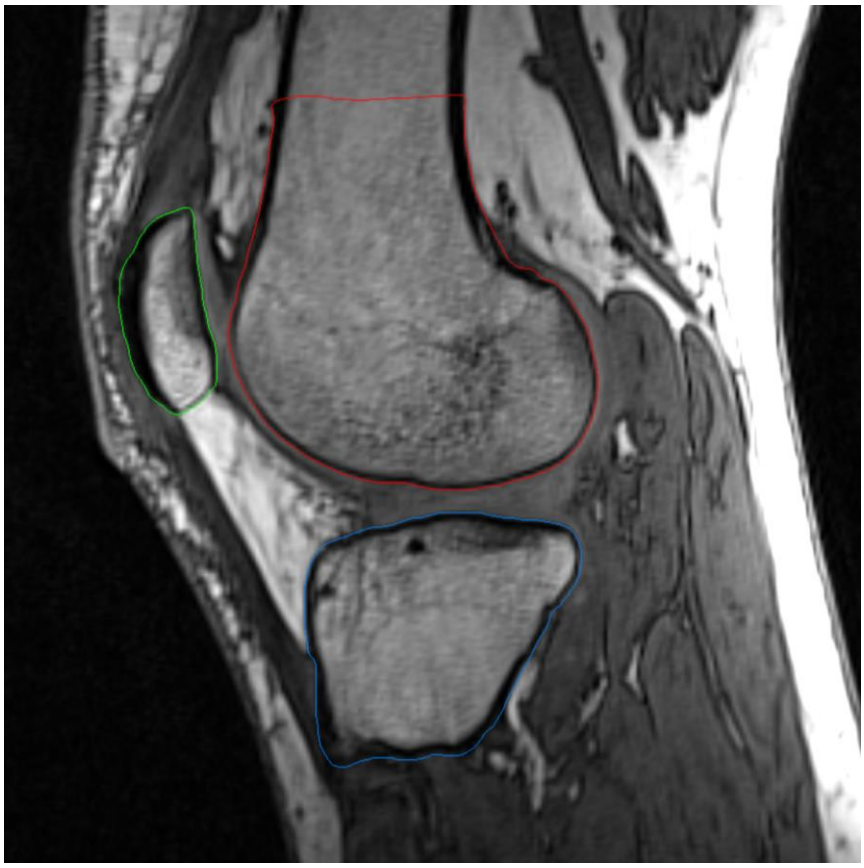


(i) (ii)

Forty-seven baseline images (first batch available) were manually segmented by an experienced musculoskeletal segmenter, supervised by a senior research scientist with 15 years' experience of manual segmentation (Mike Bowes). Three-dimensional surfaces of femur, tibia and patella bones were created from the image contours using a 3D signed-distance function, and geometrically smoothed. These surfaces were used to produce shape models and active appearance models (AAM), a form of shape model, which is capable of automatically segmenting the bones in unseen images. AAMs have been established as a technique, which can be used for segmentation of knee joint surfaces with submillimetre accuracy [545, 551].

These AAMS were used to search all available acromegaly images, generating 3D surfaces of femur, patella and tibia. AAMs record each 3D surface as a set of anatomically corresponded points (landmarks), which can be used to take measurements at consistent points on a 3D surface, and to combine groups of knees as needed. For this study, measurements included bone area in anatomical regions, and 3D distance between the bones. To make the results comparable with published work, the correspondence points from previously published models had to be transferred onto the acromegaly segmentations, allowing then to use identical regions and measurement points. This was achieved by fitting the points of a standard model to each 3D acromegaly bone, by first registering the surfaces (align them in 3D), and then project the points from the standard models onto the acromegaly surfaces, along normals projected from each model point from the standard model.

Figure 4.2. Manual segmentation of bone: femur (red), tibia (blue), patella (green)



4.3.6.ii. Bone Area (tAB)

Bone area is calculated within anatomical regions on the femur, tibia and patella. The bone surface measured refers to the bone, which is covered by cartilage, and included the following anatomical sites: medial femur, lateral femur, medial tibia, lateral tibia, medial patella, lateral patella, trochlear medial femur and trochlear lateral femur. The medial femur / medial trochlear femur and lateral femur / lateral trochlear femur boundaries were defined as a line on the bone corresponding to the anterior edge of the medial or lateral meniscus in the mean model. The medial trochlear femur / lateral trochlear femur boundary was defined as the centre of the trochlear groove in the mean model. Joint space width was calculated as the minimum inter-bone distance between the femoral and tibial bones. The different regions, where bone area was measured are shown in Figure 4.3.

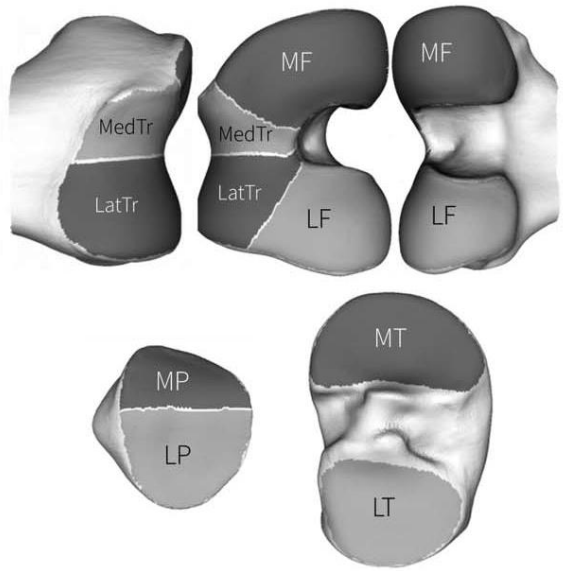


Figure 4.3. Schematic representation of the anatomical bone areas measured in this study. MF: medial femur; LF: lateral femur; MT: medial tibia; LT: lateral tibia; MP: medial patella; LP: lateral patella; MedTr: medial trochlear femur; LatTr: lateral trochlear femur.

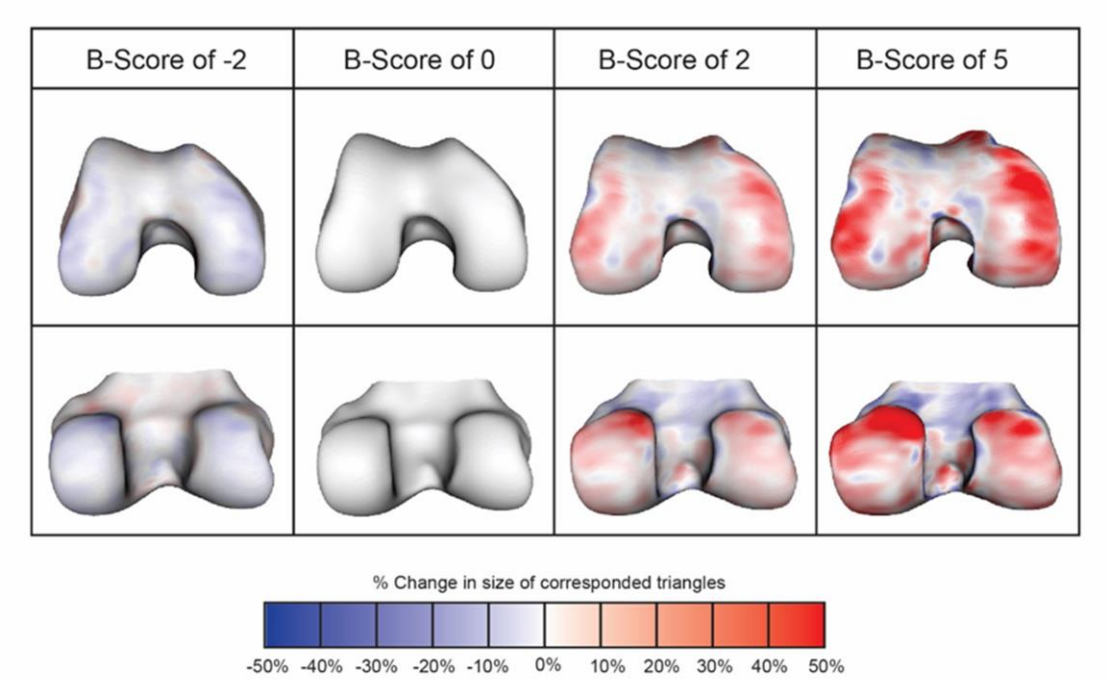
4.3.6.iii. B-score

B-score is based on 3D femur bone shape, which changes in a characteristic way with osteoarthritis, involving the growth of an osteophyte "crust" accompanied with spreading and flattening of the subchondral bone. An "OA vector" was constructed, being the line between the mean shape of a population who do not have radiographic OA, and the mean shape of

population that do have radiographic OA. The distance along this line records how much OA shape is present in the knee. The origin is set at the distance along the vector of the mean of the non-OA group (by sex), and 1 unit of B-score is defined as 1 standard deviation of the non-OA group. This is equivalent to the T-score in osteoporosis, which is defined using a healthy group to set zero, with units of standard deviation [551]. Therefore higher (more positive) values of B-score point towards the bone shape of the OA population.

The range of B-scores in the non-OA population Group was defined as the 95% confidence limits of B-scores in this group, being ±1.96. Therefore, B-scores between -1.96 and +1.96 were considered to represent the normal (reference) range, with values >1.96 indicating presence of OA. Examples of femur bone shape at different B-scores and a heat map of the areas that change with increasing B-score are shown in Figure 4.4.

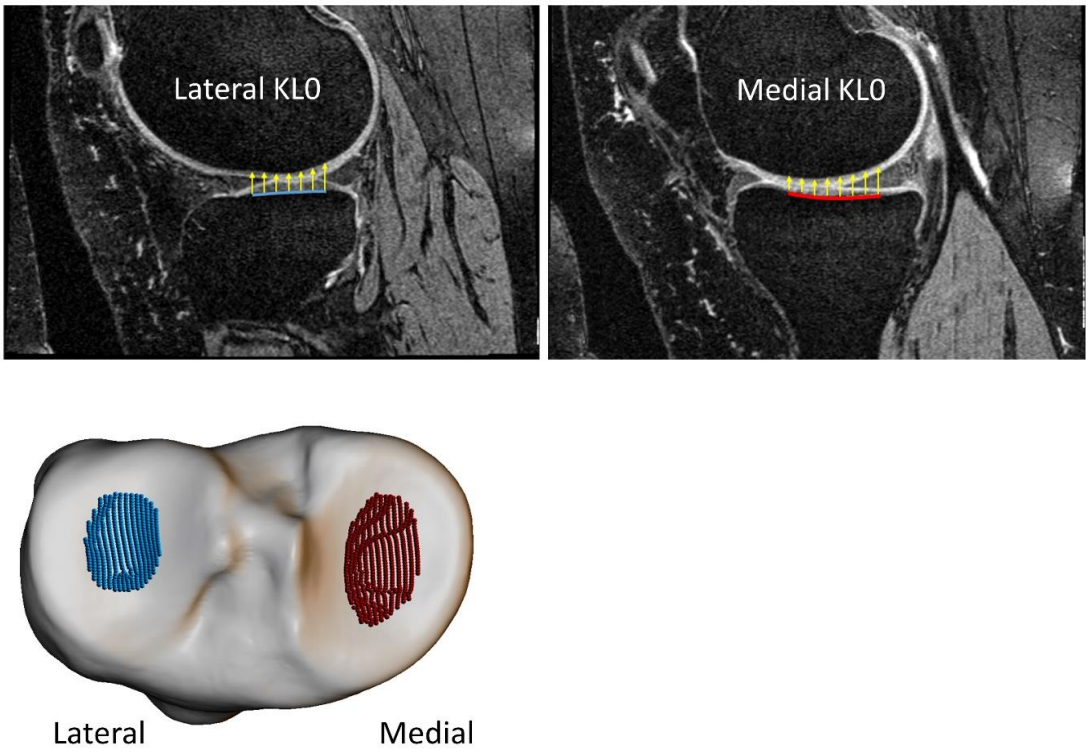
Figure 4.4. Figure shows change in shape for the anterior femur (top row) and posterior femur (bottom row), for various B-scores. Red indicates increase in the size of bone area (locally calculated, based on anatomically corresponded triangles from the shape model), and blue indicates decrease in size (locally); scale shows percentage in area size change of each triangle.



4.3.6.iv. 3D joint space width

The 3D distance between the femur and tibia bones, in the central region of the medial and lateral condyles, was measured in the MRI vibe image as follows (Figure 4.5). Measurements were taken from the tibia of aligned knees (aligned so that y axis is the patient's superior-inferior direction), using the central medial tibial region (red in figure) and lateral (blue), with yellow arrows denoting the direction in which measurements are taken. Measurements were taken at each correspondence point in the model (correspondence points from which measurements are taken are shown as red circles (medial) and blue circles (lateral). The distance from the tibial point to the femoral surface was recorded, and summarised as mean thickness and various percentiles (each region is composed of around 500 points).

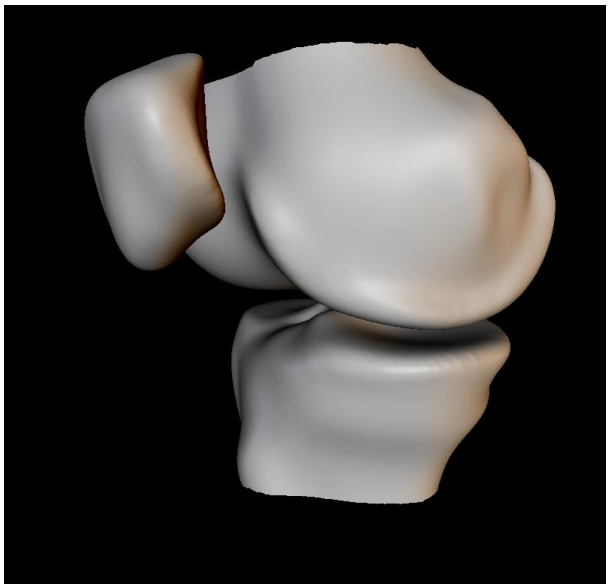
Figure 4.5. Methodology applied in the measurement of 3D joint space width. Representative slices of the sagittal image are shown in the two panes in the centre of the medial and lateral condyles of a representative knee with a Kellgren-Lawrence (KL) grade of 0.



4.3.6.v. Bone volume

Bone volume is calculated as the Gaussian volume of the femur, tibia and patella bone surfaces, in mm^3. The extent of the bone shafts included in the calculation is shown in the Figure 4.6.

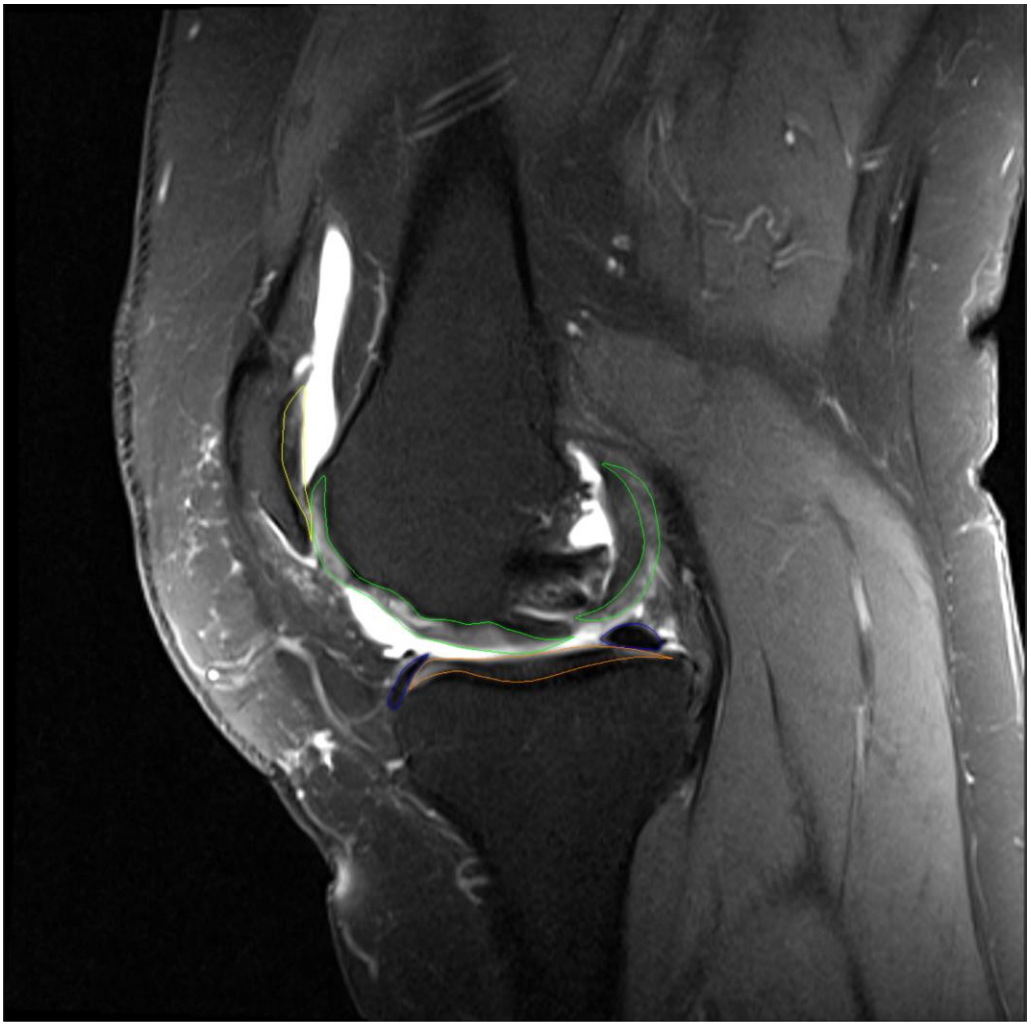
Figure 4.6. Bone volume calculation; extent of volume included in calculation



4.3.6.vi. Cartilage thickness measurement

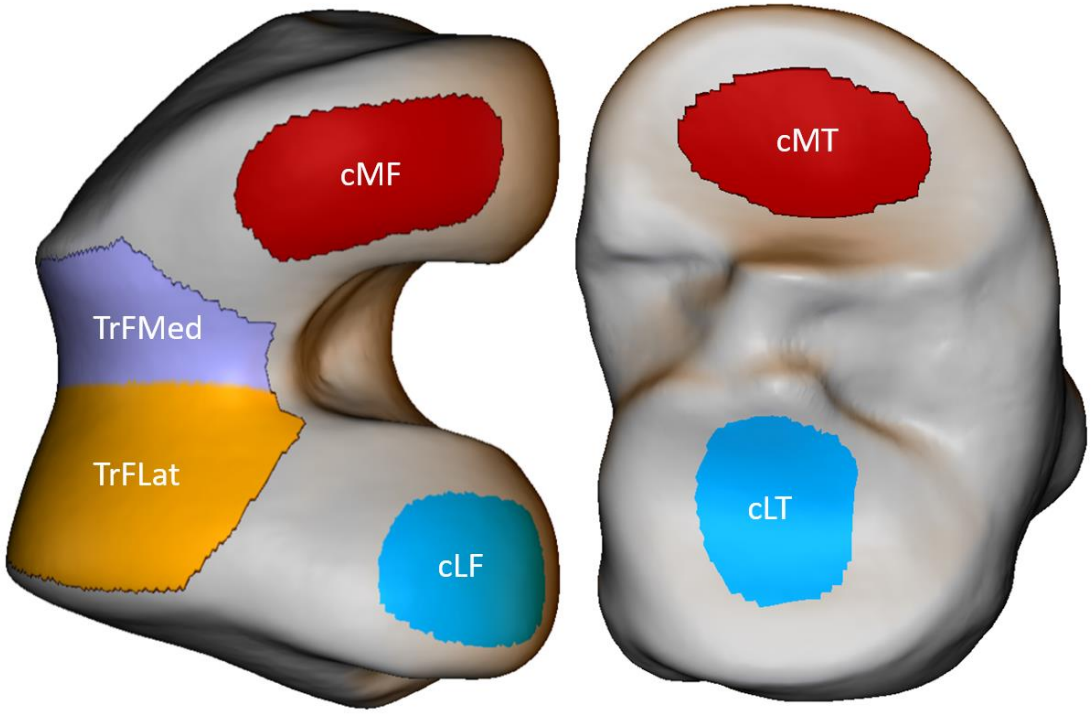
As the cartilage was so poorly defined in the vibe images, the lower resolution (3mm slice thickness) proton-density turbo spin echo sequences were used instead. Manual segmentation of the cartilage was done by the method described previously for bone segmentation. Cartilage measurements were performed using the Anatomically Corresponded Regional Analysis of Cartilage (ACRAC) method [553, 554], which uses model points on the bone from which measurements are taken (as in 3D joint space width). The bone model surface was provided by searching the TSE images using standard Imorphics (fat-saturated) AAMs of femur, tibia and patella. The regions used for measurement are shown in Figure 4.7. Two recent publications have shown that this method of automated cartilage segmentation is indistinguishable from expert manual segmentation, and is highly accurate and repeatable [555, 556]. Figure 4.8 shows the different regions where cartilage thickness was measured.

Figure 4.7. Manual segmentation of cartilage and menisci femur (green), tibia (orange), patella (yellow) and meniscus (blue).



Analysis by Imoprhics was done on anonymised images by a single operator. No clinical information regarding the presence or severity of joint symptoms or acromegaly disease activity was provided. Measurements of joint space width, bone area at the different anatomical regions, B-score and cartilage thickness were measured separately for the right and left knee in each study participant. The average value of the measurements for the right and left knee was used in the statistical analysis, when patients with acromegaly were compared with controls from the OAI.

Figure 4.8. Schematic representation of the different cartilage regions in the knee joint where cartilage thickness was measured.



cMF	Central Medial Femur
cMT	Central Medial Tibia
MFMT	cMF + cMT
cLF	Central Lateral Femur
cLT	Central Lateral Tibia
LFLT	cLF + cLT
TrFMed	Medial Trochlear Femur
TrFLat	Lateral Trochlear Femur

4.3.7. Statistical analysis

Descriptive data are presented as mean and standard deviation, or medial and interquartile range for parametric and non-parametric data respectively. Non-paired t-test for continuous variables and Mann-Whitney U-test (for variables which failed normality test) were used to assess the difference in the values between different comparison groups. The Chi-square or Fisher Exact test was used to compare proportions between the different study groups. Comparisons in bone shape, joint space width and cartilage thickness between patients with acromegaly and controls were also performed following adjustment for age, gender, height and weight, using analysis of covariance test (ANCOVA).

Multiple linear regression analysis was also performed. The model included B-score as dependent variable and age, gender, height, weight, GH or IGF-1 levels at diagnosis and the different treatment modalities (pituitary surgery or radiotherapy or medical therapy) as independent variables. A second model was used with 3-D joint space width as dependent variable and age, gender, height, weight, GH or IGF-1 levels at diagnosis, duration of disease activity and GH or IGF-1 or disease activity status at the time of the study as independent variables.

For the longitudinal arm of the study, comparison in JSW and bone shape parameters were performed using the paired t-test or the Wilcoxon signed rank test were used for parametric and non-parametric variables respectively.

A P value of <0.05 was considered statistically significant. Statistical analysis was performed using the statistics software “SigmaPlot” (Systat Software Inc. London, UK).

4.4. RESULTS

Patients' recruitment

A total of 74 consecutive patients with a previous history of acromegaly were approached to take part to the study. Nine patients declined to participate due to difficulties attending for the study visit (i.e. unable to take time off work, living far away); two were unable to tolerate the MRI scan due to claustrophobia; while in one patient MRI was contraindicated due to a pacemaker. Sixty-two patients were recruited to the study and had MRI scans of the knee joints. Due to technical reasons, scans for two patients were not available at the end of the study and they were excluded. Eventually, 60 patients were included to the study and the results analyses; 58 patients had scans of both knee joints, while in the remaining 2 only one knee was scanned due to previous knee replacement to the other joint.

4.4.1. Entire patients' cohort (N=60) vs. controls (N=300)

4.4.1.i. Patients' characteristics – acromegaly related history

Twenty-nine male and thirty-one female patients with acromegaly completed the cross-sectional part of the study. The mean age was 54.8±12.9 years and the mean BMI was 31.3±5.9 kg/m^2. With the exception of one patient who was diagnosed with a GHRH-secreting bronchial carcinoid causing acromegaly, the remaining cases of acromegaly were due to GH-secreting pituitary adenomas. Based on the American Endocrine Society clinical practice guidelines 2014 [311], 26 patients (43.3%) had active acromegaly at the time of the study (GH >1mcg/L and IGF-1 above the age-specific reference range or dichotomous GH/IGF-1 results), while the remaining 34 patients (56.7%) were in remission (GH <1mcg/L and IGF-1 within the age-specific reference range). For the entire patient cohort, the median GH and IGF-1 at the time of the study were 0.51 (IQR 0.2-1.3) mcg/L and 91.5 (IQR 59.6-127.7) % of the upper limit of normal (ULN), respectively.

The mean age at diagnosis of acromegaly was 41.5±12.9 years and the estimated age at the onset of symptoms of acromegaly was 34.6±13.1 years, based on patients' reports. The

median duration of active disease (estimated from the time on onset of symptoms until the time disease remission was achieved) was 11.7 years (25-75% IQR 5.25-21.7 years), while the median duration of disease remission was calculated at 2.58 years (25-75% IQR 0-9 years). Fifty-one patients (85%) had trans-sphenoidal surgery, while 9 patients did not have any surgical intervention to their pituitary gland. Of the patients who underwent trans-sphenoidal surgery, 35 had surgery once, 15 had surgery twice, while 1 patient required surgery three times. Twenty-six patients (43.3%) had radiotherapy to the pituitary gland, which in all cases was an adjuvant therapy to pituitary surgery. External beam radiotherapy 45 Gy in 25 fractions was delivered in 21 cases (80.7%); 4 patients (15.4%) had gamma-knife radiotherapy to the GH-secreting pituitary adenoma; while the remaining 1 patient (3.9%) had both external beam and gamma-knife radiotherapy for persistent acromegaly.

The majority of patients (n=51, 85%) had been on medical therapy during the course of their disease. Forty-four patients were treated with somatostatin analogues (either Octreotide LAR or Lanreotide) and sixteen patients received treatment with dopamine agonists (cabergoline or bromocriptine), in conjuction with somatostatin analogues in half of the cases and as monotherapy in the remaining eight. One patient was on treatment with the GH receptor antagonist Pegvisomant, which was used in combination with Octreotide LAR. Overall, 35 patients (58.3%) were on medical therapy at the time of the study.

Following multi-modality treatment for acromegaly, 32 patients (53.3%) had developed hypopituitarism; 31 patients (51.7%) had LH/FSH deficiency; 20 patients (33.3%) had ACTH deficiency; 17 patients (28.3%) had TSH deficiency; and 4 patients (6.7%) had diabetes insipidus (ADH deficiency). All patients with hypopituitarism had been on long-term (for at least 3 months prior to the study) appropriate hormone replacement therapy, with the exception of post-menopausal women with gonadotrophin deficiency, in which oestrogen replacement therapy was not clinically indicated.

With regards to the initial tumour size, 21 patients (35%) were found to have a macroadenoma (>10mm), 10 patients (16.7%) had a mesoadenoma (5-10mm), 6 patients (10%) had a microadenoma (<5mm), in one case the patient had pituitary hyperplasia rather

than adenoma secondary to ectopic GHRH secretion from a bronchial carcinoid causing pituitary stimulation, while in 22 cases (36.7%) no data were available regarding the initial pituitary tumour size. Table 4.1 summarises the data presented above.

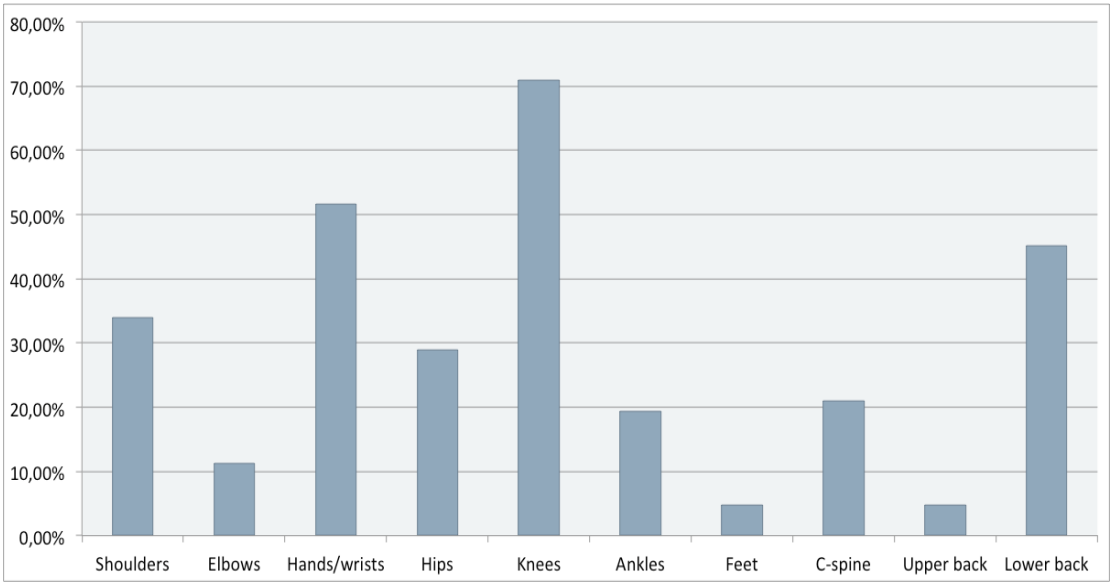
Table 4.1. Summary of the acromegaly-related history of the acromegaly study group.

Characteristic	**Acromegaly Group (N=60)**
Gender	
• Male	29 (48.3%)
• Female	31 (51.7%)
Age (years)	54.8±12.9
BMI (kg/m^2)	31.3±5.9
Treatment modalities	
• Trans-sphenoidal surgery	51 (85%)
• Cranial radiotherapy	26 (43.3%)
• Medical therapy	51 (85%)
Pituitary dysfunction following therapy	
• LH/FSH deficiency	31 (51.7%)
• ACTH deficiency	20 (33.3%)
• TSH deficiency	17 (28.3%)
• ADH deficiency	4 (6.7%)
Biochemical disease status	
• Median duration of active acromegaly (years)	11.7 (5.25-21.7)
• Median duration of disease remission (years)	2.58 (0-9)
• Patients with active disease	26 (43.3%)
• Patient with disease remission	34 (56.7%)
• Median GH at the time of the study (mcg/L)	0.51 (0.2-1.3)
• Median IGF-1 (% ULN) at the time of the study	91.5 (59.6-127.7)

4.4.1.ii. Patients' characteristics – arthropathy related history

The vast majority of patients (88.3%, n=53) reported pain in at least one joint area. The knees were the most commonly affected area with 71.7% of patients reporting knee pain, followed by the small joints of hand (wrists and fingers), affected in 51.7% of cases. The order of the remaining affected joint areas, in reducing frequency of reported pain was: lower back (45%); shoulders (33.3%); hips (28.3%); cervical spine and ankles (20%); elbows (11.7%); feet/toes (5%). Figure 4.9 summarises these results. In most cases, the pain was bilateral, with the frequency of bilateral arthralgia varying from 65% in the shoulders to 100% in the feet/toes.

Figure 4.9. Prevalence of arthralgia in the different anatomical areas, as reported by patients with acromegaly (N=60).



The knee was most commonly reported as the most painful joint area (28.3% of cases), followed by the lower back (15%) and the hips (14.2%). Figure 4.10 shows the distribution of joint areas, which were reported as the most painful joint site by the patients. Only 7 patients (11.7%) reported absence of pain in all joint areas; 6 patients (10%) reported pain in one joint; while 47 patients (78.3%) reported pain in more than one joint areas (Figure 4.11).

Figure 4.10. Localisation of most painful joint as reported by patients with acromegaly (N=60).

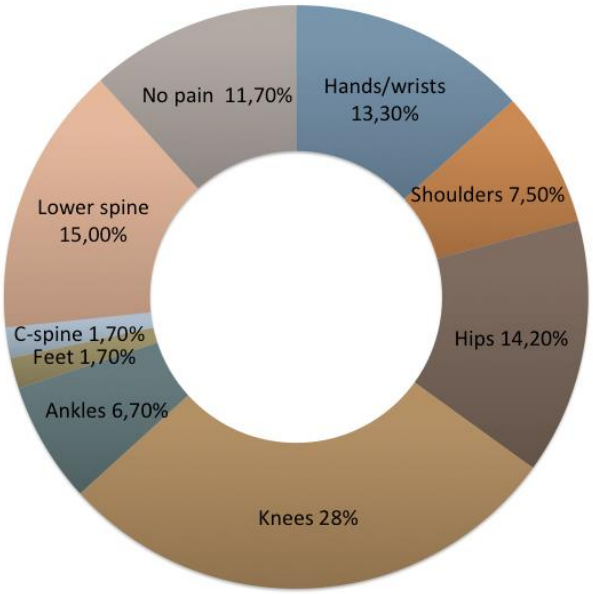
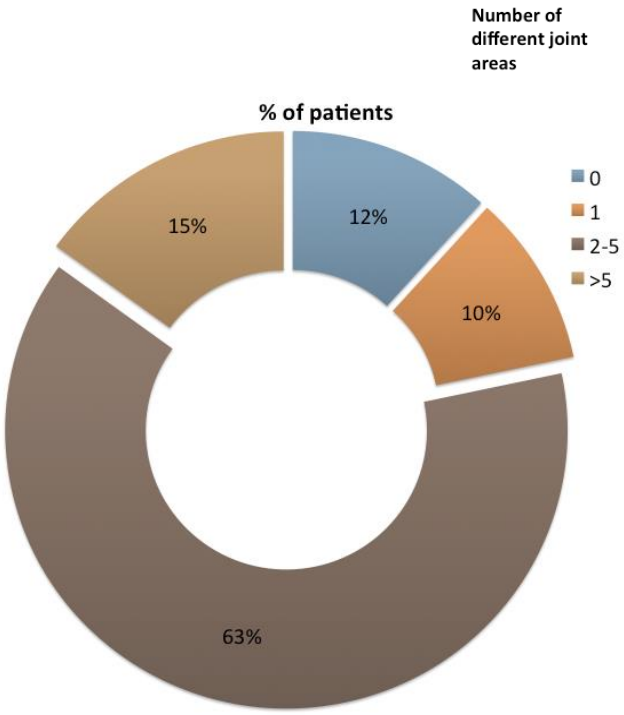


Figure 4.11. Number of different anatomical areas affected by joint pain in a single patient, based on patients reported outcomes (N=60).

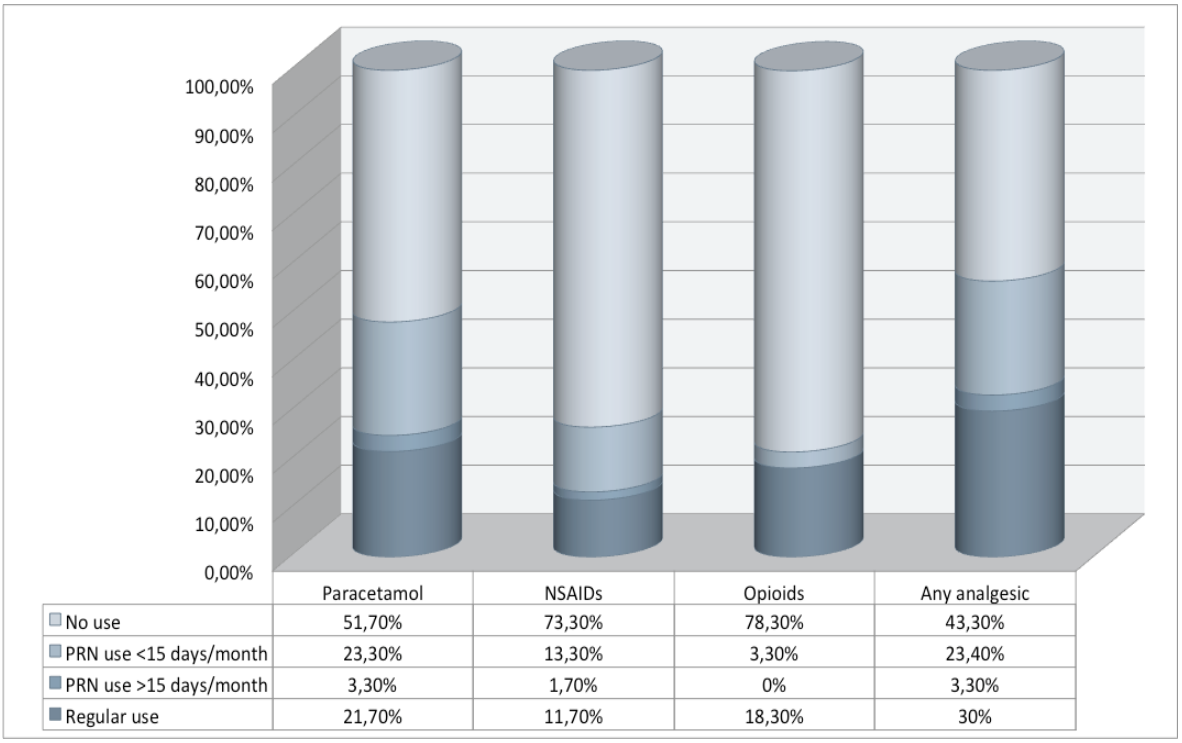


For patients who reported knee pain, the median duration of pain was 4 years (IQR 2-7 years) in the right knee and 3.75 years in the left knee (IQR 2-8 years). 58.3% and 63.3% of patients reported that they experienced pain in the right and left knee respectively in the last month from the time of the study visit, with approximately 40% of those reporting pain for more than 20 days in the last month (45.7% right knee pain and 39.5% left knee pain). Additionally, almost a third of patient reported that had knee pain for more than 3 months in the last year from time of the study visit (31.7% right knee pain and 36.7% left knee pain).

Notably, 15 patients (25%) had previously undergone joint replacement surgery due to arthropathy (6 patients had arthroplasty in one joint; 7 patients had surgery in two joints; while 2 patients had surgery in three joints). The hip was the most common site of joint replacement surgery in 11.7% of patients, followed by the lumbar spine (5%), the cervical spine (3.3%), the knee and the shoulder (2.5% each). The median age of patients at the time of their first joint surgery was 48 years (IQR 40-63 years).

Regarding the use of systemic oral analgesia for joint pain, 34 patients (56.7%) reported requiring analgesia; 18 on a regular basis (30%) and 16 patients on a PRN basis (26.7%). Paracetamol was the most frequently used analgesic, with 29 patients (48.3%) taking it either on a PRN or a regular basis. Non-steroidal anti-inflammatory medications (NSAIDs) were the second most commonly used category of analgesics (n=16 patients, 26.7%), followed by opiates (n=13 patients, 21.7%). Two patients reported using other forms of analgesia (amitriptyline or gabapentin). Of the patients who were taking analgesics, 15 patients were on a monotherapy for analgesia, 12 patients were using two different forms of analgesia, while 7 patients were on triple oral analgesic therapy (Figure 4.12). In addition to oral analgesia, 14 patients (23.3%) were using other topical therapies for localized knee pain, most commonly topical NSAIDs (n=10, 16.7%), followed by physiotherapy (n=7, 11.7%), intra-articular steroid injections (n=5, 8.3%), acupuncture (n=2, 3.3%), self-reported exercise (n=2, 3.3%) and walking aids (n=2, 3.3%).

Figure 4.12. Use of different types of analgesia in patients with acromegaly for symptomatic relief from joint pain. Results are presented as percentages of the total number of patients (N=60).



	Paracetamol	NSAIDs	Opioids	Any analgesic
No use	51,70%	73,30%	78,30%	43,30%
PRN use <15 days/month	23,30%	13,30%	3,30%	23,40%
PRN use >15 days/month	3,30%	1,70%	0%	3,30%
Regular use	21,70%	11,70%	18,30%	30%

4.4.1.iii. Control Group

Three hundred individuals were selected through propensity score matching from the OAI database (145 males and 155 females). The mean age was 57.1±9.2 years (p=0.11 when compared with patients) and although there was no significant difference in BMI [patients median BMI 30.3 (27-32.2) kg/m^2 vs. controls median BMI 29.0 (26.2-31.8) kg/m^2; p=0.08], acromegaly patients had significantly higher weight compared with controls [patients median weight 88.5 (79.8-105.1) kg vs. controls median weight 84.1 (74.1-95) kg; p=0.024]. No difference in the height between the two groups was found [patients median height 1.71 (1.63-1.81) m vs. controls median height 1.69 (1.61-1.77) m; p=0.22].

As it is possible that individuals with early stages of OA might have been included in the control group described above, a second control group was extracted from the OAI database, comprising of individuals who did not demonstrate any features of OA based on serial MRI

scans over a period of 4 years. No matching with patients was performed on this occasion. This control group, also referred as "OA-free group", included 886 individuals (391 males and 495 females, p=0.62 when compared with the male:female ratio in the patient group) of a mean age of 59±8.9 years (p<0.001 compared with patients). The individuals of the OA-free group had significantly lower height, weight and BMI compared with acromegaly patients [Height: patients median height 1.71 (1.63-1.81) m vs. OA-free controls median height 1.68 (1.61-1.76) m, p=0.03; Weight: patients median weight 88.5 (79.8-105.1) kg vs. OA-free controls median weight 75.8 (74.5-87.9) kg, p<0.001; BMI: patients median BMI 30.3 (27-32.2) kg/m^2 vs. OA-free controls median BMI 26.5 (24-29.8) kg/m^2, p<0.001]. Table 4.2 summarises the comparison of the basic characteristics between patients and controls.

Table 4.2. Comparison of the basic clinical characteristics between patients and control groups.

Characteristic	**Acromegaly patients (N=60)**	**Propensity matching controls (N=300)**	**p-value**	**Acromegaly patients (N=60)**	**OA-free controls (N=886)**	**p-value**
Male:Female ratio	29:31	145:155	1.0	29:31	391:495	0.62
Mean age (years)	54.8±12.9	57.1±9.2	0.11	54.8±12.9	59±8.9	<0.001
Median height (m)	1.71 (1.63-1.81)	1.69 (1.61-1.77)	0.22	1.71 (1.63-1.81)	1.68 (1.61-1.76)	0.03
Median weight (kg)	88.5 (79.8-105.1)	84.1 (74.1-95)	0.024	88.5 (79.8-105.1)	75.8 (74.5-87.9)	<0.001
Median BMI (kg/m^2)	30.3 (27-32.2)	29.0 (26.2-31.8)	0.08	30.3 (27-32.2)	26.5 (24-29.8)	<0.001

4.4.1.iv. Bone Shape

Patients with acromegaly had wider joint space medially compared with controls (6.25 ± 1.23 vs. 5.75 ± 0.88 mm, p<0.001); however no statistically significant difference was observed in the joint space width laterally (patients: 7.64 ± 1.48 mm; controls: 7.40 ± 0.86 mm; p=0.086). Femoral bone volume was similar in both groups (patients: 149396 ± 32038 mm^3; controls: 146400 ± 31935 mm^3; p=0.5). Larger bone area was found amongst patients with acromegaly compared with controls in the lateral patella (750.1 ± 145.1 vs. 701.8 ± 112.2 mm^2, p=0.004), medial patella (589.3 ± 110.9 vs. 549.9 ± 85 mm^2, p=0.002) and medial tibia (1230.8 ± 196.4 vs. 1174.7 ± 184.6 mm^2, p=0.034). No difference in the bone area was found in the lateral femur, lateral tibia, medial femur, lateral and medial trochlear femur areas. Median B-score was overall higher for patients compared with controls [1.41 (0.72 - 2.50) vs. 0.81 (-0.02 – 1.92), p<0.001].

Following adjustment for age, gender, height and weight, using analysis of covariance (ANCOVA), similar differences were observed between the two groups with significantly increased medial joint space width [patients: 6.21 (95% CI 6.03-6.40) mm; controls 5.78 (95% CI 5.70-5.87) mm; p<0.001]; lateral patella bone area [patients: 739 (95% CI 719-759) mm^2; controls: 706 (95% CI 697-715) mm^2; p=0.003]; medial patella bone area [patients: 581 (95% CI 566-596) mm^2; controls 553 (95% CI 546-560) mm^2; p=0.001]; medial tibia bone area [patients: 1217 (95% CI 1191-1243) mm^2; controls: 1184 (95% CI 1172-1195) mm^2; p=0.02] and B-score [patients: 1.70 (95% CI 1.32-2.08); controls: 1.01 (95% CI 0.84-1.18)] for patients with acromegaly compared with controls. Results from ANCOVA analysis are presented in Table 4.3.

Table 4.3. Comparison of knee bone shape between patients with acromegaly (N=60) and controls from the OAI (N=300), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. 3DJSW: 3-dimensional joint space width; LF tAB: lateral

femur bone area; LP tAB: lateral patella bone area; LT tAB: lateral tibia bone area; MF tAB: medial femur bone area; MP tAB: medial patella bone area; MT tAB: medial tibia bone area; TrFLat tAB: trochlear femur lateral bone area; TrFMed tAB: trochlear femur medial bone area; CI: confidence interval; SE: standard error.

	All Patients (N=60)		**All Controls (N=300)**		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
Femoral Bone Volume (mm^3)	146362 ± 1950	142527-150197	147007 ± 864	145308-148706	0.76
3DJSW Lateral (mm)	7.52 ± 0.11	7.31-7.74	7.43 ± 0.05	7.33-7.52	0.43
3DJSW Medial (mm)	6.21 ± 0.10	6.03-6.40	5.78 ± 0.04	5.70-5.87	**<0.001**
LF tAB (mm^2)	1732 ± 20.8	1692-1774	1743 ± 9.2	1725-1761	0.65
LP tAB (mm^2)	739 ± 10.1	719-759	706 ± 4.5	697-715	**0.003**
LT tAB (mm^2)	943 ± 10.7	922-964	936 ± 4.7	927-945	0.55
MF tAB (mm^2)	2477 ± 28	2422-2532	2433 ± 12.4	2408-2456	0.15
MP tAB (mm^2)	581 ± 7.7	566-596	553 ± 3.4	546-560	**0.001**
MT tAB (mm^2)	1217 ± 13.1	1191-1243	1184 ± 5.8	1172-1195	**0.02**
TrFLat tAB (mm^2)	1283 ± 13.6	1256-1309	1284 ± 6.0	1273-1296	0.90
TrFMed tAB (mm^2)	677 ± 7.7	662-692	694 ± 3.4	687-700	0.056
B-score	1.70 ± 0.19	1.32-2.08	1.01 ± 0.09	0.84-1.18	**0.001**

4.4.1.v. Cartilage thickness

Cartilage thickness was significantly higher in all areas of the knee joint in patients with acromegaly compared with controls as assessed by ANCOVA and following adjustment for age, gender, height and weight. This included increased thickness of femoral and tibial cartilages both medially and laterally, as well as increased combined mean femorotibial cartilage thickness at the medial [patients: 5.58 (95% CI 5.4-5.76) mm; controls 3.87 (95% CI 3.79-3.95) mm; p<0.001] and lateral [patients: 6.87 (95% CI 6.68-7.05) mm; controls: 4.99 (95% CI 4.91-5.07) mm; p<0.001] aspect of the knee joint. Cartilage measurements are summarized in Table 4.4.

Table 4.4. Comparison of cartilage measurements at the different anatomical sites of the knee joint between patients with acromegaly (N=60) and controls from the OAI (N=300), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. CI: confidence interval; SE: standard error.

	All patients (N=60)		**All Controls (N=300)**		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
Medial Femoral cartilage (mm)	2.8 ± 0.05	2.71-2.89	1.9 ± 0.02	1.86-1.94	**<0.001**
Medial Tibial cartilage (mm)	2.78 ± 0.05	2.68-2.88	1.96 ± 0.02	1.92-2.01	**<0.001**
Lateral Femoral cartilage (mm)	3.35 ± 0.04	3.27-3.43	2.2 ± 0.02	2.16-2.23	**<0.001**
Lateral Tibial (mm)	3.52 ± 0.07	3.37-3.66	2.8 ± 0.03	2.73-2.86	**<0.001**
Medial Femorotibial cartilage (mm)	5.58 ± 0.09	5.4-5.76	3.87 ± 0.04	3.79-3.95	**<0.001**
Lateral Femorotibial cartilage (mm)	6.87 ± 0.1	6.68-7.05	4.99 ± 0.04	4.91-5.07	**<0.001**

4.4.2. Comparison between patients with B-score ≥2 (n=21) and patients with B-score with B-score <2 (n=39)

4.4.2.i. Baseline patients' characteristics

The cut-off of 2 in the B-score was used to divide patients with acromegaly into two subgroups: those with a B-score <2 (Group 1), representing patients without significant evidence of knee OA on MRI and those with B-score ≥2 (Group 2), who were considered to have significant degree of knee OA. There were no significant differences between the groups with regards to mean age, male to female ratio, height, weight and BMI. Results are shown in Table 4.5.

Table 4.5. Comparison between patients with MRI evidence of OA based on B-score ≥2 (n=21) and patients without evidence of OA (B-score <2, n=39).

Characteristic	**Acromegaly Patients with B-score <2 (n=39)**	**Acromegaly Patients with B-score ≥2 (n=21)**	**P-value**
Age (years)	53.5 ± 12.1	57.4 ± 14.1	0.27
Male/Female ratio	19/20	10/11	0.85
Height (m)	1.71 ± 0.1	1.73 ± 0.12	0.54
Weight (kg)	90.8 ± 20.6	94.5 ± 18.6	0.52
BMI (kg/m^2)	31.0 ± 5.7	31.8 ± 6.3	0.61

4.4.2.ii. Acromegaly-related history

Patients from both subgroups were of similar age at the time of diagnosis of acromegaly and at the estimated time of onset of acromegaly-related symptoms. Baseline median growth hormone levels at the time of diagnosis and before any treatment were significantly higher for patients with B-score ≥2 [Group 1: median GH 6.6 mcg/L (IQR 4.1-17); Group 2: median GH 39 mcg/L (IQR 5.4-57.8); p=0.015]; however no difference was found in the IGF-1 values at

diagnosis (IGF-1 was expressed as % of ULN). GH and IGF-1 values at the time of the study were similar in both patient groups.

A larger proportion of patients with B-score ≥2 required more than one pituitary surgeries (Group 1: 15.4%; Group 2: 47.6%; p=0.016), with a similar trend for radiotherapy to the pituitary gland (Group 1: 33.3%; Group 2: 61.9%; p=0.06). There was no significant difference in the proportion of patients who required medical therapy during the disease course (Group 1: 79.5%; Group 2: 95.2%; p=0.14).

With regards to differences in the outcomes of treatment in the two patient groups, there was a higher proportion of patients with hypopituitarism following treatment in the group of patients with B-score ≥2 (Group 1: 43.6%; Group 2: 71.4%; p=0.04). There was no difference in the ratio of patients with active acromegaly and those in remission at the time of study between the two groups [Group 1: 17/22 (active disease/remission, respectively); Group 2: 9/12 (active disease/remission, respectively); p=0.83)]. Median duration of disease remission was similar in the two groups; however there was a trend for longer median duration of active disease in patients with B-score ≥2 [Group 1: 9 years (IQR 5-20.5); Group 2: 16 years (IQR 8.5-23.3); p=0.1].

A summary of the comparative data regarding the acromegaly-related history in the two patient subgroups is provided in Table 4.6.

Table 4.6. Comparison of the acromegaly-related history between acromegaly patients with B-score <2 (n=39) and patients with B-score ≥2 (n=21).

Characteristic	**Patients with B-score <2** (n=39)	**Patients with B-score ≥2** (n=21)	**P-value**
Age at diagnosis of acromegaly (years)	40.8 ± 11.5	42.7 ± 15.4	0.6
Age at onset of acromegaly symptoms (years)	34.8 ± 10.4	34.3 ± 17.2	0.9
Median duration of active acromegaly (years)	9 (5-20.5)	16 (8.5-23.3)	0.1
Median duration of acromegaly remission (years)	2.58 (0-8.2)	2.8 (0-9.3)	0.78
Median GH at diagnosis (mcg/L)	6.6 (4.1-17)	39 (5.4-57.8)	**0.015**
Median IGF-1 at diagnosis (%ULN)	238 (167-385)	310 (206-404)	0.36
Median GH at the time of the study (mcg/L)	0.56 (0.28-1.33)	0.5 (0.2-1.3)	0.67
Median IGF-1 at the time of the study (%ULN)	90.9 (68.3-133.1)	94.8 (56.9-116.2)	0.64
Number of pituitary surgeries (0/ 1/ >1)	8 / 25 /6	1 / 10/ 10	**0.016**
Radiotherapy (yes/no)	13 / 26	13 / 8	0.06
Medical therapy for acromegaly (yes/no)	31 / 8	20 / 1	0.14
Disease status at the time of the study (active/remission)	17 / 22	9 / 12	0.83
Use of medical therapy at the time of the study (yes/no)	23 / 16	12 / 9	0.89
Degree of hypopituitarism (none/ 1 hormone deficit/ >1 hormone deficits)	22 / 4 / 13	6 / 7 / 8	**0.04**

Consistent with the results presented above, a multiple linear regression analysis model with B-score as dependent variable and age, gender, height, weight, GH levels at diagnosis and the different treatment modalities (pituitary surgery or radiotherapy or medical therapy) as independent variables showed a positive correlation between B-score and increasing number of pituitary surgeries (co-efficient 0.96, p=0.02), radiotherapy (co-efficient 1.27, p=0.04) and use of medical treatment (i.e. somatostatin analogues and/or dopamine agonists) in the management of acromegaly (co-efficient 1.78, p=0.047). No correlation was found between B-score and IGF-1 values at diagnosis, as well as GH and IGF-1 values at the time of study or current disease activity status.

4.4.2.iii. Arthropathy patient-reported outcomes

There was no significant difference in the proportion of patients who reported pain at any joint site of the upper, lower limbs and spine between patients with a B-score ≥2 and those with a B-score of <2. However a larger proportion of patients with B-score ≥2 had previously had joint replacement surgery for OA (Group 1: 12.8%; Group 2: 47.6%; p=0.009). Although similar proportion of patients reported knee pain in the groups (Group 1: 64.1%; Group 2: 85.7%; p=0.14), duration of median knee pain was significantly longer for patients with B-score ≥2 [Group 1: 1.5 years (IQR 0-5); Group 2: 4 (IQR 1.75-10); p=0.01].

There was no difference in the use of paracetamol and NSAIDs between the two groups, however a larger proportion of patients with B-score ≥2 were using opiates at least at a PRN basis (Group 1: 12.8%; Group 2: 38.1%; p=0.045). When all different categories of analgesics were combined together, there was a trend towards more regular use of analgesics in patients with B-score ≥2 [Group 1: 20/11/8 (no use/PRN use/regular use of analgesics respectively); Group 2: 6/5/10 (no use/PRN use/regular use of analgesics respectively); p=0.08]. There was no significant difference in the proportion of patients who were using topical treatments for knee pain. Results are summarized in Tables 4.7 and 4.8.

Table 4.7. Comparison of reported joint pain between acromegaly patients with B-score <2 (n=39) and patients with B-score ≥2 (n=21).

Joint area	**Patients with B-score <2** (n=39)	**Patients with B-score ≥2** (n=21)	**P-value**
Hands / fingers (pain/no pain)	19 / 20	9 / 12	0.87
Wrist (pain/no pain)	10 / 29	0 / 21	0.01
Elbow (pain/no pain)	6 / 33	1 / 20	0.4
Shoulder (pain/no pain)	11 / 28	9 / 12	0.39
Hip (pain/no pain)	12 / 27	5 / 16	0.79
Knee (pain/no pain)	25 / 14	18 / 3	0.14
Ankle (pain/no pain)	9 / 30	3 / 18	0.5
Feet (pain/no pain)	2 / 37	1 / 20	1.0
Neck (pain/no pain)	10 / 29	2 / 19	0.18
Upper back (pain/no pain)	2 / 37	0 / 21	0.54
Lower back (pain/no pain)	16 / 23	11 / 10	0.59
Median number of painful joint areas	3 (2-4)	3 (2-4)	0.95
Median duration of knee pain (years)	1.5 (0-5)	4 (1.75-10)	**0.01**
Previous joint replacement surgery (yes/no)	5 / 34	10 / 11	**0.009**

Table 4.8. Comparison of the use of analgesia between acromegaly patients with B-score <2 (n=39) and patients with B-score ≥2 (n=21).

	Patients with B-score <2 (n=39)	**Patients with B-score ≥2** (n=21)	**P-value**
Use of paracetamol (yes/no)	18 / 21	11 / 10	0.85
Use of NSAIDs (yes/no)	10 / 29	6 / 15	0.95
Use of opioids (yes/no)	5 / 34	8 / 13	0.045
Number of different analgesics used (0 / 1 / >1)	20 / 7 / 12	6 / 8 / 7	0.14
Frequency of use of analgesia (no use / PRN / regular use)	20 / 11 / 18	6 / 5 / 10	0.08
Use of topical treatment (yes/no)	7 / 32	7 / 14	0.31

4.4.2.iv. Bone Shape

Patients with B-score ≥2 had numerically increased joint space width compared with patients with B-score <2, both at the medial (Group 1: 6.08 ± 1.14 mm; Group 2: 6.58 ± 1.37 mm; p=0.11) and lateral aspect of the knee joint (Group 1: 7.39 ± 1.33 mm; Group 2: 8.08 ± 1.66 mm; p=0.08), however this did not reach the level of statistical significance. Femoral bone volume was not significantly different between the two groups (Group 1: 145253 ± 29674 mm^3; Group 2: 157090 ± 35488 mm^3; p=0.17).

Increased bone area was found in almost all sites of the knee joint in patients with B-score ≥2, including lateral patella (Group 1: 712 ± 134 mm^2; Group 2: 821 ± 141 mm^2; p=0.005); lateral tibia (Group 1: 925 ± 142 mm^2; Group 2: 1015 ± 171 mm^2; p=0.033); medial femur (Group 1: 2427 ± 331 mm^2; Group 2: 2664 ± 444 mm^2; p=0.023); medial patella (Group 1: 560 ± 100 mm^2; Group 2: 644 ± 112 mm^2; p=0.004); medial tibia (Group 1: 1192 ±177 mm^2; Group 2: 1304 ± 211 mm^2; p=0.034) and medial trochlear femur (Group 1: 670 ± 87 mm^2;

Group 2: 724 ± 110 mm^2; p=0.032). As expected median B-score was significantly higher in Group 2 compared with Group 1 (Group 1: 0.93 (IQR 0.38-1.4); Group 2: 2.7 (2.4-4.6); p<0.001.

Similar results to the above were observed after adjusting for age, gender, height and weight. There was a trend towards wider joint space in patients with B-score ≥2, both at the medial [Group 1: 6.08 mm (95% CI 5.79-6.37); Group 2: 6.58 (95% CI 6.18-6.98); p=0.054] and the lateral [Group 1: 7.44 mm (95% CI 7.1-7.9); Group 2 7.99 mm (95% CI 7.52-8.47); p=0.07], although without reaching statistical significance. With the exception of lateral and medial trochlear femur, bone area was significantly increased at all other sites of the knee joint for patient with B-score ≥2, including the lateral femur, lateral patella, lateral tibia, medial femur, medial patella, and medial tibia. B-score was significantly higher in Group 2. Bone shape results following ANCOVA analysis are presented in Table 4.9.

Multiple linear regression analysis, using 3-D joint space width as dependent variable and age, gender, height, weight, duration of active acromegaly, GH or IGF-1 at diagnosis, GH or IGF-1 or disease activity status at the time of the study, showed positive correlation between JSW and higher GH levels at diagnosis (coefficient 0.013, p=0.015), height (coefficient 3.55, p=0.01), weight (coefficient 0.02, p=0.004) and male gender (coefficient 0.74, p=0.02), which were all independent positive predictive factors of increased JSW. No correlation was found between JSW and IGF-1 at diagnosis and also between JSW and GH or IGF-1 levels or disease activity status at the time of the study.

Table 4.9. Comparison of knee bone shape between acromegaly patients with B-score <2 (n=39) and patients with B-score ≥2 (n=21), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. 3DJSW: 3-dimensional joint space width; LF tAB: lateral femur bone area; LP tAB: lateral patella bone area; LT tAB: lateral tibia bone area; MF tAB: medial femur bone area; MP tAB: medial patella bone area; MT tAB: medial tibia bone area; TrFLat tAB: trochlear femur lateral bone area; TrFMed tAB: trochlear femur medial bone area; CI: confidence interval; SE: standard error.

	Patients with B-score <2 (N=39)		**Patients with B-score ≥2** (N=21)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
Femoral Bone Volume (mm^3)	148294 ± 3047	142185-154403	151444 ± 4208	143006-159881	0.56
3DJSW Lateral (mm)	7.44 ± 0.17	7.1-7.79	7.99 ± 0.24	7.52-8.47	0.07
3DJSW Medial (mm)	6.08 ± 0.14	5.79-6.37	6.58 ± 0.2	6.18-6.98	0.054
LF tAB (mm^2)	1727 ± 23	1681-1773	1815 ± 31.8	1751-1879	**0.03**
LP tAB (mm^2)	723 ± 13.5	696-750	801 ± 18.6	764-838	**0.001**
LT tAB (mm^2)	937 ± 13.8	909-964	992 ± 19.1	954-1031	**0.025**
MF tAB (mm^2)	2456 ± 37.7	2380-2532	2611 ± 52.1	2506-2715	**0.022**
MP tAB (mm^2)	568 ± 11	546-590	628 ± 15.1	598-658	**0.003**
MT tAB (mm^2)	1204 ± 15.8	1172-1236	1281 ± 21.8	1237-1324	**0.007**
TrFLat tAB (mm^2)	1292 ± 16.1	1260-1325	1315 ± 22.2	1270-1359	0.43
TrFMed tAB (mm^2)	675 ± 9.8	655-695	709 ± 13.6	681-736	0.055
B-score	0.81 ± 0.2	0.41-1.20	3.61 ± 0.27	3.07-4.15	**<0.001**

4.4.2.v. Cartilage thickness

Following adjustment for age, gender, height and weight, patients with B-score ≥2 had significantly increased mean cartilage thickness in most joint sites, including the medial tibial, lateral femoral, and lateral tibial sites. The combined medial and lateral femorotibial cartilage thickness was also significantly increased in patients with B-score ≥2. No difference in cartilage thickness was found at the medial femur, medial and lateral trochlear femur. Results are shown in Table 4.10.

Table 4.10. Comparison of cartilage measurements at the different anatomical sites of the knee joint between acromegaly patients with B-score <2 (n=39) and patients with B-score ≥2 (n=21), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. LF: lateral femur; LT: lateral tibia; MF: medial femur; MT: medial tibia; TrFLat: trochlear femur lateral; TrFMed: trochlear femur medial; CI: confidence interval; SE: standard error.

	Patients with B-score <2 (N=39)		**Patients with B-score ≥2** (N=21)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
MF cartilage (mm)	2.77 ± 0.1	2.57-2.98	2.93 ± 0.14	2.65-3.21	0.38
MT cartilage (mm)	2.58 ± 0.09	2.41-2.75	3.19 ± 0.12	2.96-3.43	**<0.001**
LF cartilage (mm)	3.25-0.07	3.1-3.39	3.65 ± 0.1	3.45-3.84	**0.002**
LT cartilage (mm)	3.24 ± 0.12	3.0-3.47	4.12 ± 0.16	3.82-4.44	**<0.001**
TrFLat cartilage (mm)	2.98 ± 0.06	2.85-3.11	3.02 ± 0.08	2.85-3.19	0.73
TrFMed cartilage (mm)	3.1 ± 0.07	2.96-3.24	3.22 ± 0.1	3.03-3.43	0.32
Medial Femorotibial cartilage (mm)	5.35 ± 0.17	5.0-5.7	6.12 ± 0.23	5.66-6.59	**0.012**
Lateral Femorotibial cartilage (mm)	6.48 ± 0.16	6.17-6.79	7.78 ± 0.21	7.36-8.2	**<0.001**

4.4.3. Comparison between patients with B-score ≥2 (n=21) and their respective controls (n=105)

4.4.3.i. Bone shape

There was no significant difference in the femoral bone volume between the two groups (patients: 157090 ± 35488 mm^3; controls: 144727 ± 30724 mm^3; p=0.1). Joint space width was increased in patients with acromegaly both in the lateral (patients: 8.08 ± 1.66 mm; controls: 7.33 ± 0.88 mm; p=0.003) and medial (patients 6.58 ± 1.38 mm; controls: 5.77 ± 0.9 mm; p<0.001) areas. Bone area was also larger for patients at the lateral patella (patients: 820.7 ± 140.7 mm^2; controls: 701.5 ± 111.9 mm^2; p<0.001), lateral tibia (patients: 1015.1 ± 171.3 mm^2; controls: 926.8 ± 143 mm^2; p=0.014), medial femur (patients: 2663.8 ± 444.1 mm^2; controls: 2424.6 ± 353.6 mm^2; p=0.008); medial patella (patients: 644.4 ± 112 mm^2; controls: 549.6 ± 84.3 mm^2; p<0.001) and medial tibia (patients: 1303.7 ± 213.4 mm^2; controls: 1177.8 ± 178.5 mm^2; p=0.005). No statistically significant difference to the size of the bone area was observed at the lateral femur, lateral and medial trochlear femur areas. As expected, B-score was significantly higher in patients compared with controls [patients: median B-score 2.7 (IQR 2.4 - 4.6); controls: median B-score 0.8 (IQR -0.01- 1.98); p<0.001].

Similar results were found after adjustment for age, gender, height and weight, with increased joint space width both in the lateral [patients: 7.86 (95% CI 7.45-8.27) mm; controls: 7.38 (95% CI 7.20-7.55) mm; p=0.035] and medial [patients: 6.6 (95% CI 6.23-6.96) mm; controls: 5.76 (95% CI 5.60-5.92) mm; p<0.001] areas for knee joint. Patients also had larger bone areas at the lateral patella, lateral tibia, medial femoral, medial patella and medial tibia areas. Similar to the unadjusted results, no significant difference in the femoral bone volume and size of bone area at the lateral femur, medial and lateral trochlear femur areas was found. B-score was significantly higher in patients compared with controls [patients: 3.65 (95% CI 3.01-4.28); controls: 1.1 (95% CI 0.82-1.37); p<0.001]. Results of ANCOVA analysis are shown in Table 4.11.

Table 4.11. Comparison of knee bone shape between acromegaly patients with B-score ≥2 (n=21) and their respective controls from the OAI (n=105), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. 3DJSW: 3-dimensional joint space width; LF tAB: lateral femur bone area; LP tAB: lateral patella bone area; LT tAB: lateral tibia bone area; MF tAB: medial femur bone area; MP tAB: medial patella bone area; MT tAB: medial tibia bone area; TrFLat tAB: trochlear femur lateral bone area; TrFMed tAB: trochlear femur medial bone area; CI: confidence interval; SE: standard error.

	Patients with B-score ≥2 (N=21)		**Controls** (N=105)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
Femoral Bone Volume (mm^3)	148442 ± 3381	141747-155137	146456 ± 1465	143555-149357	0.60
3DJSW Lateral (mm)	7.86 ± 0.2	7.45-8.27	7.38 ± 0.09	7.20-7.55	**0.035**
3DJSW Medial (mm)	6.60 ± 0.18	6.23-6.96	5.76 ± 0.08	5.60-5.92	**<0.001**
LF tAB (mm^2)	1772 ± 31.1	1710-1833	1739 ± 13.5	1712-1766	0.34
LP tAB (mm^2)	786 ± 16.1	754-818	708 ± 7.0	695-722	**<0.001**
LT tAB (mm^2)	974 ± 17.3	940-1009	935 ± 7.5	920-950	**0.04**
MF tAB (mm^2)	2564 ± 48.9	2467-2660	2445 ± 21.2	2403-2487	**0.029**
MP tAB (mm^2)	618 ± 12.2	593-642	555 ± 5.3	544-565	**<0.001**
MT tAB (mm^2)	1256 ± 21.3	1214-1298	1187 ± 9.2	1169-1206	**0.004**
TrFLat tAB (mm^2)	1294 ± 21.3	1252-1336	1284 ± 9.2	1265-1302	0.67
TrFMed tAB (mm^2)	696 ± 12.9	671-722	695 ± 5.6	684-706	0.93
B-score	3.65 ± 0.32	3.01-4.28	1.10 ± 0.14	0.82-1.37	**<0.001**

4.4.3.ii. Cartilage thickness

Mean cartilage thickness between the two groups was compared following adjustment for age, gender, height and weight. Significantly increased cartilage thickness was found in acromegaly patients with B-score ≥2 compared with their respective controls in both lateral and medial femur and tibia. Results are summarized in Table 4.12.

Table 4.12. Comparison of cartilage measurements at the different anatomical sites of the knee joint between acromegaly patients with B-score ≥2 (n=21) and their respective controls from the OAI (n=105), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. LF: lateral femur; LT: lateral tibia; MF: medial femur; MT: medial tibia; CI: confidence interval; SE: standard error.

	Patients with B-score ≥2 (N=21)		**Controls** (N=105)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
MF cartilage (mm)	2.88 ± 0.1	2.69-3.07	1.89 ± 0.04	1.81-1.97	<**0.001**
MT cartilage (mm)	3.1 ± 0.1	2.91-3.28	1.96 ± 0.04	1.88-2.04	<**0.001**
LF cartilage (mm)	3.55 ± 0.07	3.42-3.69	2.18 ± 0.03	2.13-2.24	<**0.001**
LT cartilage (mm)	4.01 ± 0.13	3.76-4.26	2.75 ± 0.06	2.64-2.86	<**0.001**
Medial Femorotibial cartilage (mm)	5.97 ± 0.18	5.62-6.32	3.85 ± 0.08	3.7-4.0	<**0.001**
Lateral Femorotibial cartilage (mm)	7.56 ± 0.16	7.24-7.88	4.93 ± 0.07	4.79-5.07	<**0.001**

4.4.4. Comparison between patients with B-score <2 (n=39) and their respective controls (n=195)

4.4.4.i. Bone shape

No difference in femoral bone volume or in the size of bone area at any anatomical site of the knee joint was observed between patients and controls. B-scores were also similar in the two groups [patients: medical B-score 0.93 (IQR 0.38 – 1.4); patients: median B-score 0.86 (IQR -0.06 – 1.95); p=0.84]. Patients had wider joint space, but only in the medial aspect of the joint (patients: 6.08 ± 1.14 mm; controls: 5.78 ± 0.7 mm; p=0.033).

Following adjustment for age, gender, height and weight, patients with acromegaly continued to have significantly higher joint space width medially compared with controls [patients: 6.04 (95% CI 5.83-6.26) mm; controls: 5.79 (95% CI 5.69-5.88) mm; p=0.028]. Patients had smaller bone area at the medial trochlear femur compared with controls [patients: 664 (95% CI 645-682) mm^2; controls: 694 (95% CI 685-702) mm^2; p=0.003]; however no difference was observed in the femoral bone volume, B-score or in the size of the bone area at any other site (Table 4.13).

Table 4.13. Comparison of knee bone shape between acromegaly patients with B-score <2 (n=39) and their respective controls from the OAI (n=195), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. 3DJSW: 3-dimensional joint space width; LF tAB: lateral femur bone area; LP tAB: lateral patella bone area; LT tAB: lateral tibia bone area; MF tAB: medial femur bone area; MP tAB: medial patella bone area; MT tAB: medial tibia bone area; TrFLat tAB: trochlear femur lateral bone area; TrFMed tAB: trochlear femur medial bone area; CI: confidence interval; SE: standard error.

	Patients with B-score <2 (N=39)		**Controls** (N=195)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
Femoral Bone Volume (mm^3)	144951 ± 2425	140173-149729	147362 ± 1078	145238-149486	0.37
3DJSW Lateral (mm)	7.33 ± 0.13	7.08-7.59	7.46 ± 0.06	7.35-7.57	0.38
3DJSW Medial (mm)	6.04 ± 0.11	5.83-6.26	5.79 ± 0.05	5.69-5.88	**0.028**
LF tAB (mm^2)	1699 ± 25.7	1648-1749	1748 ± 11.4	1725-1770	0.082
LP tAB (mm^2)	709 ± 12.1	686-733	705 ± 5.4	695-716	0.76
LT tAB (mm^2)	921 ± 12.8	895-946	938 ± 5.7	927-949	0.21
MF tAB (mm^2)	2416 ± 32.9	2352-2481	2429 ± 14.6	2400-2458	0.73
MP tAB (mm^2)	558 ± 9.2	540-576	553 ± 4.1	545-561	0.62
MT tAB (mm^2)	1188 ± 15.8	1157-1219	1183 ± 7.0	1169-1197	0.77
TrFLat tAB (mm^2)	1269 ± 17	1236-1303	1286 ± 7.6	1272-1301	0.36
TrFMed tAB (mm^2)	664 ± 9.2	645-682	694 ± 4.1	685-702	**0.003**
B-score	0.73 ± 0.22	0.29-1.17	0.95 ± 0.1	0.76-1.15	0.36

4.4.4.ii. Cartilage thickness

Similar to patients with B-score ≥2, acromegaly patients with B-score <2 had significantly increased mean cartilage thickness in both medial and lateral femur and tibia areas, compared with their respective controls and following adjustment for age, gender, height and weight. Results are summarized in Table 4.14.

Table 4.14. Comparison of cartilage measurements at the different anatomical sites of the knee joint between acromegaly patients with B-score <2 (n=39) and their respective controls from the OAI (n=195), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. LF: lateral femur; LT: lateral tibia; MF: medial femur; MT: medial tibia; CI: confidence interval; SE: standard error.

	Patients with B-score <2 (N=39)		**Controls** (N=195)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
MF cartilage (mm)	2.76 ± 0.05	2.66-2.86	1.91 ± 0.02	1.87-1.96	**<0.001**
MT cartilage (mm)	2.61 ± 0.06	2.49-2.72	1.97 ± 0.03	1.92-2.02	**<0.001**
LF cartilage (mm)	3.23 ± 0.05	3.14-3.33	2.2 ± 0.02	2.16-2.24	**<0.001**
LT cartilage (mm)	3.24 ± 0.08	3.08-3.4	2.82 ± 0.04	2.75-2.89	**<0.001**
Medial Femorotibial cartilage (mm)	5.37 ± 0.1	5.17-5.57	3.88 ± 0.05	3.79-3.97	**<0.001**
Lateral Femorotibial cartilage (mm)	6.47 ± 0.11	6.25-6.69	5.02 ± 0.05	4.93-5.12	**<0.001**

4.4.5. Comparison between patients with B-score <2 (n=39) and the OA-free control group (n=886)

4.4.5.i. Bone shape

A total of 886 individuals, who did not have any evidence of knee OA based on serial MRI scans over a 5-year follow-up period, were identified from the OAI database. These are referred as the "OA-free" group and were compared with acromegaly patients with a B-score of <2. Patients with acromegaly were younger in age compared with the controls of the OA-free group and had significantly higher weight and BMI. There was no difference in the male/female ratio or in height between the two groups. The results have previously been reported in Table 4.2 of this Chapter.

Patients had smaller joint space width medially (patients: 6.08 ± 1.14 mm; ultra-controls: 6.27 ± 0.95 mm; p=0.01) but not laterally compared with the OA-free group. Patients had significantly larger bone area at the lateral patella (patients: 712 ± 134 mm^2; OA-free group: 669 ± 112 mm^2; p=0.02); medial femur (patients: 2427 ± 331 mm^2; OA-free group: 2310 ± 363 mm^2; p=0.048), medial patella (patients: 560 ± 100 mm^2; OA-free group: 523 ± 85 mm^2; p=0.008), with a trend for the medial tibia area (patients: 1192 ± 177 mm^2; OA-free group 1135 ± 181 mm^2; p=0.057). B-score was significantly higher for the patient group [patients: median B-score 0.93 (IQR 0.38-1.4); OA-free group: median B-score 0.05 (IQR -0.6 – 0.68); p<0.001].

Similar differences were observed following adjustment for age, gender, height and weight, using ANCOVA analysis. Medial joint space width was higher for the individuals of the OA-free group compared with patients. However, patients had larger bone area at the lateral patella, medial patella, medial tibia, with a trend for the medial femoral area. B-score was significantly higher in patients compared with the OA-free group. Results of the ANCOVA analysis are presented in Table 4.15.

Table 4.15. Comparison of knee bone shape between acromegaly patients with B-score <2 (n=39) and controls from the OAI who did not have any evidence of OA based on serial MRI scans (n=886), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. 3DJSW: 3-dimensional joint space width; LF tAB: lateral femur bone area; LP tAB: lateral patella bone area; LT tAB: lateral tibia bone area; MF tAB: medial femur bone area; MP tAB: medial patella bone area; MT tAB: medial tibia bone area; TrFLat tAB: trochlear femur lateral bone area; TrFMed tAB: trochlear femur medial bone area; CI: confidence interval; SE: standard error.

	Patients with B-score <2 (N=39)		**OA-free Controls** (N=886)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
Femoral Bone Volume (mm^3)	140119 ± 2340	135526-144711	141475 ± 469	140555-142394	0.57
3DJSW Lateral (mm)	7.29 ± 0.11	7.07-7.5	7.39 ± 0.02	7.34-7.43	0.37
3DJSW Medial (mm)	6.0 ± 0.13	5.75-6.25	6.28 ± 0.03	6.23-6.33	**0.035**
LF tAB (mm^2)	1661 ± 19.8	1622-1670	1665 ± 4.0	1658-1673	0.83
LP tAB (mm^2)	695 ± 10.5	675-716	670 ± 2.1	666-674	**0.017**
LT tAB (mm^2)	905 ± 9.6	886-925	894 ± 1.9	890-898	0.24
MF tAB (mm^2)	2365 ± 26.4	2313-2417	2313 ± 5.3	2303-2324	0.055
MP tAB (mm^2)	547 ± 8.0	531-563	523 ± 1.6	520-526	**0.004**
MT tAB (mm^2)	1166 ± 12.7	1141-1191	1137 ± 2.5	1132-1142	**0.023**
TrFLat tAB (mm^2)	1243 ± 14.5	1215-1272	1240 ± 2.9	1234-1246	0.82
TrFMed tAB (mm^2)	652 ± 7.6	637-666	662 ± 1.5	659-665	0.16
B-score	0.69 ± 0.17	0.36-1.02	0.018 ± 0.03	-0.05-0.08	**<0.001**

4.4.5.ii. Cartilage thickness

Medial and lateral femoral and tibial cartilage thickness was significantly increased in acromegaly patients compared with the OA-free controls. Mean cartilage thickness values between the two groups were compared after adjusting for age, gender, height and weight. A summary of the results is provided in Table 4.16.

Table 4.16. Comparison of cartilage measurements at the different anatomical sites of the knee joint between acromegaly patients with B-score <2 (n=39) and the OA-free control group (n=886), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. LF: lateral femur; LT: lateral tibia; MF: medial femur; MT: medial tibia; CI: confidence interval; SE: standard error.

	Patients with B-score <2 (N=39)		**OA-free Controls** (N=886)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
MF cartilage (mm)	2.73 ± 0.04	2.645-2.81	1.93 ± 0.01	1.91-1.94	**<0.001**
MT cartilage (mm)	2.58 ± 0.05	2.47-2.68	1.99 ± 0.01	1.97-2.02	**<0.001**
LF cartilage (mm)	3.19 ± 0.05	3.1-3.28	2.15 ± 0.01	2.14-2.17	**<0.001**
LT cartilage (mm)	3.18 ± 0.07	3.04-3.31	2.81 ± 0.01	2.78-2.84	**<0.001**
Medial Femorotibial cartilage (mm)	5.31 ± 0.09	5.13-5.48	3.92 ± 0.02	3.89-3.96	**<0.001**
Lateral Femorotibial cartilage (mm)	6.36 ± 0.1	6.17-6.55	4.97 ± 0.02	4.93-5.01	**<0.001**

4.4.6. Comparison between patients who had previously undergone joint surgery (n=15) and those who had not had joint surgery (n=45)

A total of 15 patients (25%) had previously undergone joint surgery for OA-related symptoms (also referred as Group 3). This patient subgroup was of a particular interest as it was considered to indicate presence of advanced arthropathy and therefore those individuals were compared with patients who had not previously had joint surgery (also referred as Group 4).

4.4.6.i. Baseline patients' characteristics

There was no difference between the two groups in terms of mean age and male: female ratio. Median height, weight and BMI were also similar in the two patient subgroups (Table 4.17).

Table 4.17. Comparison between patients with acromegaly with previous history of joint replacement surgery (n=15) and patient without (n=45).

Characteristic	**Patients with previous joint surgery** (n=15)	**Patients without previous joint surgery** (n=45)	**P-value**
Age (years)	57.1 ± 13.4	54.1 ± 12.8	0.44
Male/Female ratio	7/8	22/23	0.88
Height (m)	1.73 (1.64-1.84)	1.71 (1.64-1.78)	0.53
Weight (kg)	87.8 (84.2-98.7)	88.4 (76.5-103.7)	0.86
BMI (kg/m^2)	29.8 (26.6-35.8)	30 (27.2-35.2)	0.75

4.4.6.ii. Acromegaly-related history

There was no difference between the two groups in the estimated median age at the onset of symptoms of acromegaly or in the median duration of active disease. Patients who had previously undergone joint surgery had significantly higher median GH levels at the time of diagnosis of acromegaly [Group 3: 39 (6.95-55.5) mcg/L; Group 4: 6.57 (3.97-17) mcg/L;

p=0.005]; however there was no difference in the median IGF-1 values at diagnosis (Table 4.18).

Although a numerically higher proportion of patient who underwent joint surgery required multiple pituitary surgeries (Group 3: 46.7%; Group 4: 20%; p=0.09) and pituitary radiotherapy (Group 3: 53.3%; Group 4: 40%; p=0.55) for the management of acromegaly, the difference was not statistically significant (Table 4.18).

Table 4.18. Comparison of the acromegaly-related history between patients with previous history of joint replacement surgery (n=15) and patient without (n=45).

Characteristic	**Patients with previous joint surgery** (n=15)	**Patients without previous joint surgery** (n=45)	**P-value**
Age at diagnosis of acromegaly (years)	40 (29-51)	40 (33-46.5)	0.8
Age at onset of acromegaly symptoms (years)	30 (18-45)	36 (25-43)	0.3
Median duration of active acromegaly (years)	14 (6-22)	11 (5-21.5)	0.4
Median GH at diagnosis (mcg/L)	39 (6.95-55.5)	6.57 (3.97-17)	**0.005**
Median IGF-1 at diagnosis (%ULN)	247 (174-357)	278 (192.5-406)	0.45
Number of pituitary surgeries (0/ 1/ >1)	1 / 7 / 7	8 / 28 / 9	0.1
Radiotherapy (yes/no)	8 / 7	18 / 27	0.55
Medical therapy for acromegaly (yes/no)	12 / 3	39 / 6	0.68
Disease status at the time of the study (active/remission)	3 / 12	28 / 17	**0.01**
Degree of hypopituitarism (none/ 1 hormone deficit/ >1 hormone deficits)	6 / 4 / 5	22 / 7 / 16	0.6

4.4.6.iii. Bone Shape

Following adjustment for age, gender, height and weight using analysis of covariance (ANCOVA), patients who had previously undergone joint surgery had significantly increased mean joint space width both in the lateral [Group 3: 8.2 (95% CI 7.66-8.74) mm; Group 4: 7.45 (95% CI 7.14-7.76) mm; p=0.02] and medial [Group 3: 6.69 (95% CI 6.23-7.16) mm; Group 4: 6.11 (95% CI 5.84-6.37) mm; p=0.035] aspect of the knee joint, compared without previous joint surgery. There were no significant differences in the femoral bone volume and the size of the femur, tibia and patella bone areas. Mean B-scores , although numerically higher in patients with previous history of joint surgery, were not statistically dissimilar in the two groups. Results of bone shape following ANCOVA analysis are presented in Table 4.19.

4.4.6.iv. Cartilage thickness

After adjusting for age, gender, height and weight, patients with a history of previous joint surgery were found to have increased mean cartilage thickness at the lateral femorotibial site both as a combined measurement [Group 3: 7.94 (95% CI 7.44-8.44) mm; Group 4: 6.61 (95% CI 6.32-6.9) mm; p<0.001] and also as individual measurements of lateral femoral [Group 3: 3.75 (95% CI 3.52-3.97) mm; Group 4: 3.27 (95% CI 3.14-3.4) mm; p<0.001] and lateral tibial [Group 3: 4.19 (95% CI 3.81-4.57); Group 4: 3.34 (95% CI 3.12-3.56) mm; p<0.001] cartilage thickness. No difference was noted in the medial femorotibial cartilage thickness and also in the trochlear femur medial and lateral cartilages thickness between the two patient subgroups (Table 4.20).

Table 4.19. Comparison of knee bone shape between acromegaly patients with previous history of joint replacement surgery (n=15) and patient without (n=45), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. 3DJSW: 3-dimensional joint space width; LF tAB: lateral femur bone area; LP tAB: lateral patella bone area; LT tAB: lateral tibia bone area; MF tAB: medial femur bone area; MP tAB: medial patella bone area; MT tAB: medial tibia bone area; TrFLat tAB: trochlear femur lateral bone area; TrFMed tAB: trochlear femur medial bone area; CI: confidence interval; SE: standard error.

	Patients with previous joint surgery (n=15)		**Patients without previous joint surgery** (n=45)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
Femoral Bone Volume (mm^3)	150020 ± 4944	140109-159931	149188 ± 2822	143529-154847	0.88
3DJSW Lateral (mm)	8.2 ± 0.27	7.66-8.74	7.45 ± 0.15	7.14-7.76	**0.02**
3DJSW Medial (mm)	6.7 ± 0.23	6.23-7.16	6.11 ± 0.13	5.84-6.37	**0.035**
LF tAB (mm^2)	1810 ± 38.2	1733-1887	1740 ± 21.8	1670-1784	0.12
LP tAB (mm^2)	776 ± 23.4	729-823	741 ± 13.3	715-768	0.2
LT tAB (mm^2)	980 ± 22.9	934-1026	948 ± 13.1	922-975	0.24
MF tAB (mm^2)	2539 ± 63.8	2411-2667	2500 ± 36.4	2427-2573	0.6
MP tAB (mm^2)	605 ± 18.9	567-643	584 ± 10.8	562-606	0.34
MT tAB (mm^2)	1251 ± 27.1	1196-1305	1224 ± 15.5	1193-1255	0.4
TrFLat tAB (mm^2)	1314 ± 26.1	1262-1367	1295 ± 14.9	1266-1325	0.54
TrFMed tAB (mm^2)	697 ± 16.2	665-730	683 ± 9.3	665-702	0.47
B-score	2.29 ± 0.47	1.35-3.23	1.62 ± 0.27	1.08-2.16	0.23

Table 4.20. Comparison of cartilage measurements at the different anatomical sites of the knee joint between acromegaly patients with previous history of joint replacement surgery (n=15) and patient without (n=45), using analysis of covariance (ANCOVA), with adjustment for age, gender, height and weight. LF: lateral femur; LT: lateral tibia; MF: medial femur; MT: medial tibia; TrFLat: trochlear femur lateral; TrFMed: trochlear femur medial; CI: confidence interval; SE: standard error.

	Patients with previous joint surgery (n=15)		**Patients without previous joint surgery** (n=45)		**P-value**
	Mean ± SE	**95% CI**	**Mean ± SE**	**95% CI**	
MF cartilage (mm)	2.77 ± 0.16	2.44-3.09	2.85 ± 0.1	2.66-3.04	0.65
MT cartilage (mm)	3.04 ± 0.15	2.74-3.35	2.72 ± 0.09	2.54-2.90	0.07
LF cartilage (mm)	3.75 ± 0.11	3.52-3.97	3.27 ± 0.07	3.14-3.40	**<0.001**
LT cartilage (mm)	4.19 ± 0.19	3.81-4.57	3.34 ± 0.11	3.12-3.56	**<0.001**
TrFMed cartilage (mm)	3.20 ± 0.11	2.98-3.43	3.13 ± 0.07	2.99-3.26	0.56
TrFLat cartilage (mm)	2.95 ± 0.1	2.75-3.15	3.0 ± 0.06	2.89-3.12	0.63
Medial Femorotibial cartilage (mm)	5.81 ± 0.29	5.23-6.39	5.57 ± 0.17	5.23-5.91	0.48
Lateral Femorotibial cartilage (mm)	7.94 ± 0.25	7.44-8.44	6.61 ± 0.15	6.32-6.90	**<0.001**

4.4.7. Longitudinal Arm

Fifty-one patients who had previously completed the cross-sectional arm of the study attended for a second MRI scan of the knees (participation rate: 82.3%). The median time interval between the two MRI scans was 1.17 years (1.1-1.25). Of the remaining 9 patients, 6 patients were unable to attend for the second study visit due to work-related reasons (unable to take time off work); 1 patient died for reasons unrelated to acromegaly and the study; 1 patient had undergone bilateral knee replacement since the first study visit; and in 1 case MRI was contraindicated at the time of the follow up scan, due to the possibility of presence of metal foreign body in the orbital region. Due to technical reasons, scans for nine patients were not available at the end of the study and they were excluded from analysis. Eventually, 42 patients were included to the longitudinal arm of the study.

No patient underwent any further pituitary surgery or radiotherapy between first and second study visit. No changes in medications were made between the two study visits in 39 cases; one patient was started on cabergoline due to a rise in his mean GH levels based on GH day curve; in one patient the dose of sandostatin was reduced; and in one patient somatostatin analogue treatment was discontinued, in view of satisfactory biochemical disease control.

No difference in the mean lateral and medial joint space width was found between the two MRI scans. Bone area of the femur, tibia and patella at follow-up was similar to the measurements at baseline. Similarly, no difference in the median B-score was identified at follow up. In view of no significant change in joint space width and bone shape between the two MRI scans, cartilage thickness was not measured at the second study visit. The bone shape data from the longitudinal arm of the study are presented in Table 4.21.

Table 4.21. Comparison of knee bone shape in 42 patients with acromegaly at baseline and after a median follow-up of 1.17 years. Results are presented as mean values with standard deviations. Results of B-score are presented as median values with interquartile range. 3DJSW: 3-dimensional joint space width; LF tAB: lateral femur bone area; LP tAB: lateral patella bone area; LT tAB: lateral tibia bone area; MF tAB: medial femur bone area; MP tAB: medial patella bone area; MT tAB: medial tibia bone area; TrFLat tAB: trochlear femur lateral bone area; TrFMed tAB: trochlear femur medial bone area.

	Baseline MRI scan	**Follow-up MRI scan**	**P-value**
Femoral Bone Volume (mm^3)	149076 ± 32541	149410 ± 32723	0.26
3DJSW Lateral (mm)	7.58 ± 1.34	7.55 ± 1.29	0.47
3DJSW Medial (mm)	6.31 ± 1.23	6.28 ± 1.29	0.68
LF tAB (mm^2)	1772 ± 293	1775 ± 294	0.18
LP tAB (mm^2)	756 ± 153	758 ± 151	0.34
LT tAB (mm^2)	949 ± 157	950 ± 157	0.4
MF tAB (mm^2)	2509 ± 391	2512 ± 388	0.26
MP tAB (mm^2)	594 ± 117	594 ± 115	0.58
MT tAB (mm^2)	1226 ± 193	1228 ± 195	0.14
TrFLat tAB (mm^2)	1304 ± 188	1305 ± 187	0.31
TrFMed tAB (mm^2)	689 ± 100	690 ± 99	0.2
B-score	1.32 (0.59-2.32)	1.31 (0.71-2.32)	0.07

4.5. DISCUSSION

This is the first study to assess MRI bone shape in a cohort of patients with acromegaly at various stages of the disease, including both patients with active acromegaly and in remission. The main findings of the study were that patients with acromegaly have at least preserved 3-D joint space width of the knee joint, with increased width at the medial aspect of the joint compared with an age and gender-matched control group, derived from the OAI at risk of developing OA. This was due to significantly increased cartilage thickness in both lateral and medial femorotibial areas. Additionally, acromegaly patients demonstrated increased bone area at the patella and medial tibia. B-score, which is a newly proposed bio-marker for OA, was significantly higher in acromegaly patients. Differences in bone shape between patients and controls were more pronounced for the subgroup of acromegaly patients with a B-score ≥2, who demonstrated increased patella, tibia and medial femur bone area, with increased JSW both laterally and medially. However, even when "non-OA" acromegaly patients with B-score <2 were compared with a separate OA-free control group, patients had increased patella and medial tibia size and higher B-score, suggesting of altered bone shape even in these cases.

Emerging research in 3D-bone shape, measured by active appearance model, a form of statistical shape modeling, which allows automatic segmentation of bone surfaces from MR images, has provided new insights into the pathogenesis of OA. Increased subchondral bone area has been found in patients with OA [546] and has been associated with higher prevalence and severity of cartilage defects [547, 548] and risk of OA progression [549]. More recently, B-score, a marker which reflects femoral bone shape, was associated with increased severity of knee OA and more adverse clinical outcomes including joint pain and risk for knee replacement [551].

Patients with acromegaly were found to have increased bone area, primarily of the patella and secondly of the medial tibia. Increased patella bone area is not a finding that has been previously reported in OA patients and appears to be unique of patients with acromegaly. B-score was overall higher in patients with acromegaly compared with controls, indicating that

acromegaly patients are at a higher risk of progression of arthropathy, compared with age and gender matched controls from the OAI database, which included individuals at risk of developing OA. Interestingly, patients with acromegaly had preserved joint space or even increased joint space medially compared with controls, despite the higher B-score. This has been a common finding in several previous studies, which used conventional radiographs for the assessment of joint space [108, 109, 111, 511-517]. The preserved or increased joint space observed in this study can be explained by the increased cartilage thickness, which was found consistently in all areas of the knee joint in patients with acromegaly. Increased cartilage thickness is expected in acromegaly, considering the trophic effect of GH on chondrocytes [496-502] and it has been consistently reported in the literature by a number of clinical studies, using ultrasonography [518-521] and MRI for cartilage measurements [522].

B-score >1.96 (i.e. outside the 95% confidence interval of the B-score for the non-OA control group) has been considered to be suggestive of OA, with higher scores indicating greater changes in bone shape (larger bone area) and presence of more severe OA [551]. Within the acromegaly group of this study, 35% of the patients (n=21) had B-score ≥2. This patient subgroup had more pronounced changes in bone shape and increased 3D-JSW compared with their respective controls from the OAI database, with larger patella and tibia size, as well as medial femur bone area. JSW was increased both medially and laterally and cartilage thickness was also increased at all sites of the knee joint. In contrast, when acromegaly patients with B-score <2 were compared with their respective controls, there was no significant difference in bone shape or B-score. JSW was increased only medially, however cartilage thickness remained higher in the patient group compared with controls, both in the lateral and medial femorotibial areas.

Direct comparison between acromegaly patients with B-score ≥2 and those with B-score <2, showed significantly larger bone area in the former group, with increased femur, tibia and patella size. There was also a trend towards increased lateral and medial 3-D JSW, with significantly increased lateral and medial femorotibial cartilage thickness. Considering that higher B-scores indicate presence of more severe OA, one would expect that acromegaly

patients with B-score ≥2 would have narrower joint space, which is the hallmark radiological feature of OA. However, these patients had significantly wider joint space (secondary to increased cartilage thickness) when compared both with controls and acromegaly patients with B-score <2.

Comparing other clinical features between the two acromegaly subgroups, patients with B-score ≥2 were found to have significantly higher GH levels at the time of diagnosis and required higher number of interventions (surgery and radiotherapy) for the management of acromegaly, which suggest that these patients had more persistent acromegaly with an overall higher exposure to GH excess. This may explain the increased cartilage thickness and JSW in patients with B-score ≥2 and is also in-keeping with findings from previous radiological studies, which showed high prevalence of osteophytosis (which explains the larger bone area) with preserved joint space width in patients with acromegaly [108, 111]. Additionally, the preserved/increased JSW differentiates acromegalic arthropathy from classic OA, where osteophytosis is accompanied by joint space narrowing due to cartilage defects [108]. Interestingly, disease activity status and GH and IGF-1 levels at the time of the study were not different between patients with B-score greater or less than 2, implying that changes in bone shape and cartilage thickness occur at an earlier stage in the disease course and are primarily related to the degree of GH exposure during the years prior to diagnosis and treatment of acromegaly. This also suggests that the risk of more profound changes to the bone shape and subsequently to the severity of arthropathy is dependent to the degree of GH excess, making early diagnosis and successful treatment of acromegaly a focal point in the prevention of arthropathy in patients with acromegaly.

The preserved joint space may also explain the absence of any differences in the prevalence of reported pain in the different joint areas between patients with B-score ≥2 and those with B-score <2, despite more pronounced changes in bone shape in the former group. This is in-keeping with results of previous studies, which did not show any association between patients' reported outcomes and radiographic abnormalities [112]. However, patients with B-score ≥2 had longer duration of knee pain and a significantly higher prevalence of previous

joint replacement surgery. 47.6% of acromegaly patients with B-score ≥2 have previously had joint replacement surgery compared with 12.8% of patients with B-score <2 (p=0.009). This finding is consistent with the association previously described between higher B-score and risk for total knee replacement in patients with OA [551].

Median patient's age at time of first joint replacement surgery in this acromegaly cohort was 48 years (IQR 40-63), indicating that GH excess causes an accelerated form of arthropathy, which can progress to an end stage disease at a relatively young age. Further analysis showed almost equal gender distribution amongst patients who previously had joint surgery (7 male and 8 female patients). When compared with acromegaly patients without previous joint surgery there was no difference in age, height, weight and male:female ratio. Knee bone shape was similar in both patient subgroups; however, medial and lateral knee 3-D JSW was paradoxically increased in patients who had a previous history of joint replacement. Lateral (but not medial) femorotibial cartilage thickness was also increased in this patient group, as were median GH levels at the time of diagnosis, which may have accounted for the difference found in cartilage thickness and JSW. Median B-score, although numerically higher in patients with previous joint replacement (2.29 vs. 1.62), did not differ at a level of statistical significance between the two patient subgroups.

Considering that the age and gender-matched control group, derived from the OAI database, via propensity-match scoring, may have included some individuals with early stages of OA, a second control group was derived from the OAI, comprising solely of participants without any evidence of knee OA, based on serial MRI scans over a period of 4 years. This OA-free group was considered to represent a more homogeneous, healthy control population, without any knee joint pathology and was compared with the non-OA subgroup of patients with acromegaly (n=39), defined by a B-score <2. Despite differences in the baseline clinical characteristics as shown in Table 4.3 (non-OA patients were younger, with higher BMI, height and weight than the OA-free controls), analysis of bone shape following adjustment for age, gender, height and weight, showed increased patella and medial tibia size. B-score, although within normal limits, was significantly higher in acromegaly patients compared with

OA-free controls, who had a very narrow range of distribution of B-score values [patients: 0.69 (95% CI 0.36-1.02); OA-free controls 0.018 (95% CI -0.05 – 0.08); p<0.001]. Medial and lateral femorotibial cartilage thickness was significantly higher in acromegaly patients, however JSW was not increased. The above suggest that patients with acromegaly, even without evidence of overt OA on MRI, still have altered bone shape compared with healthy controls, as a result of GH excess, which may increase their risk of developing OA in the long-term, as evident by the higher B-score.

No change in bone shape and JSW was found between baseline and follow-up MRI scans, after a median period of 1.17 years. This is likely due to the short duration of follow-up in this cohort. Further prospective studies with longer duration of follow up are required to establish whether changes in bone shape occur over time in patients acromegaly, the rate these occur and whether patients with higher B-scores at baseline have higher risk of progression of arthropathy, similar to the studies in the OA population.

The main limitation of this study relates to the type of MRI sequences obtained. Due to a methodological error, a different MRI protocol was applied and only two sequence types were acquired (3D T1 Vibe sequence and 2D Proton Density TSE sequence). These sequences were different to the 3D DESS-we sequences available for the OAI controls and previously used in similar research studies. The vibe sequence, although provided high-resolution images to visualize the bone, it was of a poor quality for cartilage measurements. This was rectified using the lower resolution proton density TSE sequences and performing manual segmentation of the cartilage, which produced results similar to the automated cartilage segmentation. A second limitation of the study relates to the number of patients with acromegaly recruited, particularly in relation to intragroup analyses, as by dividing the patient group into subgroups (i.e. patients with B-score ≥2 and patients with B-score <2 or patients with history of previous joint replacement surgery and patients without previous joint surgery) there would not be adequate power to identify differences of statistical significance between subgroups.

4.6. CONCLUSIONS

In summary, this is the largest MRI study for the assessment of arthropathy in acromegaly and the first one to quantify 3D bone shape in a mixed cohort of patients with active acromegaly and disease remission. The study showed that patients with acromegaly have preserved and/or increased knee joint space width compared with controls and increased cartilage thickness in both lateral and medical femorotibial areas. Increased bone area, particularly at the patella and medial tibia, with higher B-score compared with controls was also found. Increase in bone area, involving all three bones of the knee joint (femur, tibia and patella) was more pronounced for acromegaly patients with B-score ≥2, who accounted for 35% of the entire patient cohort. Additionally, these patients had higher GH levels at diagnosis and required a higher number of therapeutic interventions during their course of acromegaly, indicating a higher degree of disease activity, which is more resistant to treatment, resulting in increased and potentially more prolonged exposure of peripheral tissues to excessive GH levels. However, despite these changes in the bone area size, which would indicate more severe arthropathy, based on MRI bone shape, patients with B-score ≥2 had increased joint space width, associated with increased cartilage thickness, which distinguishes the arthropathy in acromegaly from the classic primary osteoarthritis.

CHAPTER FIVE

ELUCIDATION OF THE POTENTIAL CARDIOVASCULAR RISK FACTORS AND CLOT DYNAMICS IN PATIENTS WITH ACROMEGALY

5.1. ABSTRACT

Objective: There remains increased cardiovascular mortality in patients with acromegaly. This study aims to evaluate whether GH/IGF-1 excess increases vascular disease by adversely affecting fibrin network characteristics.

Design: Cross-sectional study in 40 patients with acromegaly (21 males, age 53±13yrs) and 40 age/gender-matched controls.

Methods: Clot structure was analysed using a validated turbidimetric assay and fibrin networks were visualised by laser scanning confocal microscopy (LSCM). Metabolic profile parameters, body composition, plasma fibrinogen and PAI-1 were also assessed.

Results: Twenty-two patients had active acromegaly and 18 were in remission. There was no difference in qualitative patient characteristics between the two groups. Both groups had less favourable body composition and cardiovascular risk profile compared with controls. Despite no difference in clot formation and lysis parameters between the two patient groups, active disease patients had higher fibrinogen and clot maximum absorbance compared with controls, after adjusting for BMI (3.8±0.2 vs. 2.6±0.2mg/ml, $p<0.001$; and 0.39±0.02 vs. 0.33±0.01 arbitrary units, $p=0.03$, respectively). Patients in remission had higher fibrinogen compared with controls following adjustment for BMI (3.3±0.2 vs. 2.6±0.2mg/ml, $p=0.02$) but not clot maximum absorbance (0.35±0.03 vs. 0.33±0.02 arbitrary units, $p=0.6$). LSCM showed increased fibrin network density only in active disease patients, consistent with turbidimetric analysis. In addition to active disease, BMI, fat mass and skinfold thickness were associated with higher clot density and longer lysis time.

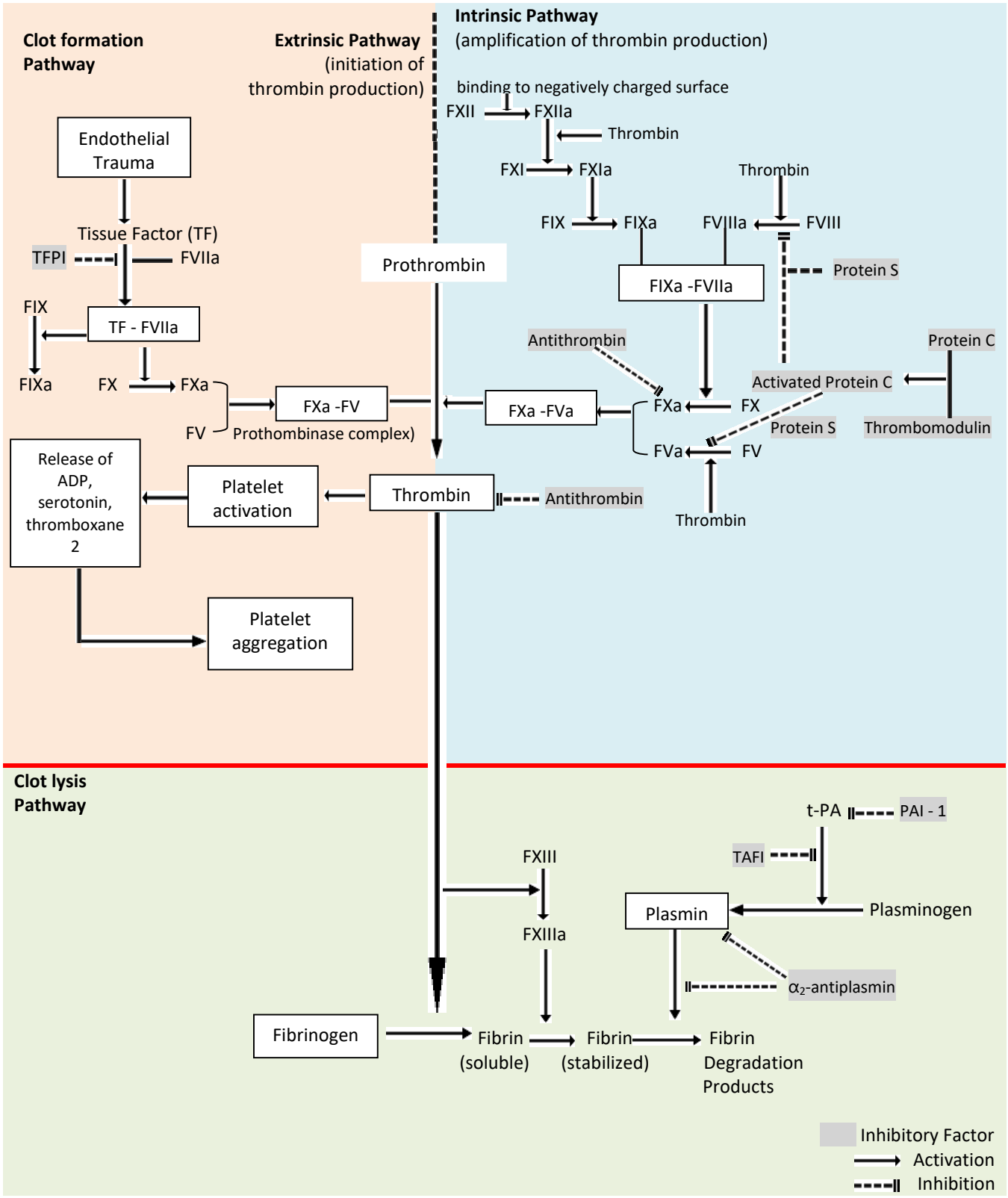
Conclusions: Patients with active acromegaly have more compact clots, thus conferring increased thrombosis risk. Prothrombotic fibrin networks may represent one mechanism for enhanced vascular risk in active acromegaly.

5.2. INTRODUCTION

5.2.1. Overview of haemostasis

The haemostatic equilibrium is the result of a complex interaction between cellular and protein phases of coagulation. Following vessel injury, platelets and coagulation factors are activated and this culminates in the formation of the fibrin network, which forms the skeleton of the blood clot. During formation of the fibrin network, the fibrinolytic system is activated to limit blood clot formation and avoid widespread vascular occlusion. The clotting process and fibrinolysis are schematically shown in Figure 5.1.

Figure 5.1. Schematic diagram of the coagulation and fibrinolysis pathways, illustrating the various interactions between promoters and inhibitors of haemostasis. Endothelial trauma is usually the trigger that activates platelets and initiates the coagulation cascade, with the production of thrombin, which augments the coagulation signals via activation of various procoagulant factors, leading to further thrombin production. Thrombin catalyses conversion of fibrinogen to fibrin. TFPI, antithrombin and the Protein C/Protein S/thrombomodulin system, on the other hand, act by inhibiting several clotting factors. Clotting formation process is terminated via activation of plasmin (through the actions of t-PA and plasminogen), which degrades fibrin, promoting clot lysis. TAFI, t-PA and α2-antiplasmin are the main inhibitors of fibrinolysis. A2-antiplasmin inhibits clot lysis both by inactivating plasmin, by forming a complex with it and also by becoming incorporated into the fibrin clot increasing its resistance to the action of plasmin. ADP, adenosine diphosphate; F, factor; a, activated; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin-activatable fibrinolysis inhibitor; TF, tissue factor; TFPI, tissue factor pathway inhibitor; t-PA, tissue-plasminogen activator.


Clot formation Pathway
Extrinsic Pathway (initiation of thrombin production)
Intrinsic Pathway (amplification of thrombin production)
binding to negatively charged surface
FXII
FXIIa
Thrombin
FXI
FXIa
Thrombin
FIX
FIXa
FVIIIa
FVIII
Endothelial Trauma
Tissue Factor (TF)
TFPI
FVIIa
Prothrombin
FIXa -FVIIa
Protein S
FIX
TF - FVIIa
Antithrombin
Protein C
Activated Protein C
FIXa
FX
FXa
FXa -FV
FXa -FVa
FXa
FX
FV
Prothombinase complex)
Protein S
Thrombomodulin
FVa
FV
Release of ADP, serotonin, thromboxane 2
Platelet activation
Thrombin
Antithrombin
Thrombin
Platelet aggregation
Clot lysis Pathway
t-PA
PAI - 1
FXIII
TAFI
Plasmin
Plasminogen
FXIIIa
α2-antiplasmin
Fibrinogen
Fibrin (soluble)
Fibrin (stabilized)
Fibrin Degradation Products
Inhibitory Factor
Activation
Inhibition

5.2.1.i. The coagulation system

The initial trigger for thrombus formation is usually an injury to the blood vessel wall. At the site of injury, collagen exposure leads to platelet adhesion and aggregation, while exposed tissue factor (TF) forms a complex with circulating factor VIIa (FVIIa) initiating blood coagulation [557]. This is the traditionally described extrinsic pathway, which, via activation of factors IX (FIX) and X (FX), leads to the formation of the factor Xa-V complex (prothrombinase complex) catalysing the conversion of prothrombin to thrombin [557]. However, it is now well accepted that division into extrinsic and intrinsic pathway is an artificial laboratory phenomenon that does not apply to the in vivo environment [558].

Generation of thrombin represents a key step in the regulation of the coagulation system as it: i) mediates platelet activation (resulting in the release of ADP, serotonin and thromboxane A2, which in turn promote further platelet recruitment), ii) catalyses conversion of fibrinogen to fibrin (a key component of stable fibrin clots), and iii) activates several coagulation factors of the intrinsic pathway [factors V, VIII and XI (FV, FVIII, FXI)], potentiating fibrin production further and amplifying the coagulation signals [557]. A number of anticoagulant factors, including Tissue Factor Pathway Inhibitor (TFPI), proteins C and S, antithrombin and thrombomodulin, inhibit different parts of the coagulation cascade, to downregulate the fibrin production and maintain the haemostatic balance. Thrombin, in addition to its procoagulant properties described above, plays also a role in the anticoagulant pathway, by activating protein C [559].

5.2.1.ii. The fibrinolytic system

Synthesized fibrin is degraded by the fibrinolytic system. Tissue plasminogen activator (t-PA) and urokinase-like plasminogen activator convert plasminogen to plasmin. Plasmin is the pivotal enzyme of the fibrinolytic system, responsible not only for the degradation of fibrin, but also for the hydrolysis of factors V, VIII, XIII and von Willebrand factor (vWF) [560]. Fibrinolytic activity is reduced by the physiological inhibitors of fibrinolysis, which include Plasminogen Activators Inhibitor-1 (PAI-1), which inhibits t-PA; α2-antiplasmin, which is

incorporated into the fibrin network to inhibit plasmin action, thereby increasing fibrinolysis resistance [561]; and thrombin-activatable fibrinolysis inhibitor (TAFI), which suppresses fibrinolysis by removing carboxy-terminal lysines from partially degrading fibrin, thus compromising plasminogen activator binding and subsequent activation of plasminogen to plasmin [562]. Table 5.1 summarises the main plasma factors that regulate coagulation and fibrinolysis.

The clinical relevance of haemostatic abnormalities has been demonstrated in a number of studies. Elevated factor VIII, IX, XI and TAFI levels, reduced TFPI, protein C and S, and certain PAI-1 polymorphisms have been associated with thrombotic conditions, such as venous thromboembolism, ischaemic stroke and myocardial infarction [563-566]. Additionally, high fibrinogen has been identified as an independent risk factor for atherosclerotic and cardiovascular disease [567]. In the light of the above, the haemostatic abnormalities associated with acromegaly cannot be considered as a benign condition.

Table 5.1. Summary of the main promoters and inhibitors of coagulation and fibrinolysis.

Procoagulant factors	Anticoagulant factors	Fibrinolytic factors	Antifibrinolytic factors
- Tissue factor (TF) - Clotting factors (FII-FXIII) - Thrombin - Von Willebrand factor (vWF)	- Tissue factor pathway inhibitor (TFPI) - Protein C/ Protein S/ Thrombomodulin system - Antithrombin	- Tissue-plasminogen activator (t-PA) - Urokinase-plasminogen activator (u-PA) - Plasmin	- Thrombin-activatable fibrinolysis inhibitor (TAFI) - Plasminogen activator inhibitor-1 (PAI-1) - Alpha-2 antiplasmin

5.2.2. Mortality and Cardiovascular risk in acromegaly

Mortality in acromegaly is discussed in more detail in Chapter 1. In summary, acromegaly has been associated with increased overall mortality compared with the general population. Two meta-analyses published in 2008 showed a mean standardised mortality ratio (SMR) of 1.72 (95% confidence interval 1.62-1.83) [474] and 1.70 (95% CI 1.5-2.0) [475] respectively. However, it has been well-recognised that overall mortality rates in acromegaly have been reducing with time, reflecting the advancements in therapeutic interventions. The reduction in mortality rates is reflected in a more recent meta-analysis from 2018, in which the SMR from clinical studies published after 2008 is not significantly higher compared with the general population (SMR 1.35, 95% CI 0.99-1.85), however meta-analysis of studies published before 2008 demonstrated an increased SMR (SMR 1.76, 95% CI 1.52-2.04) [179]. In addition to the above a separate meta-analysis of patients who achieved disease control with a final GH <2.5mcg/L and IGF-1 levels within the normal range revealed SMRs similar to the general population [179, 475]. However, based on a study from New Zealand a GH<1mcg/L appears to completely normalise mortality in acromegaly and make it indistinguishable from the general population [335].

It has been consistently shown in a number of studies that excess mortality is at least partially related to increased risk for cardiovascular and cerebrovascular disease [179, 300, 335, 352, 477]. In a recent meta-analysis, the SMR for cardiovascular death was higher in acromegaly both in studies published before 2008 (SMR 2.38, 95% CI 1.81-3.14) and also in the studies published after 2008 (SMR 1.67, 95% CI 1.35-2.05) [179]. The same applied for cerebrovascular disease. Cardiovascular mortality has been found to increase significantly with GH levels >2mcg/L and elevated IGF-1 (>2 standard deviation scores) [335]. However, in the last decade there appears to be a decrease in the number of deaths from cardiovascular causes and an increase in the number of deaths related to cancer [179, 478]. This may reflect the improvement in the longevity of patients with acromegaly, with their mortality resembling more that of the general population, rather a true association between acromegaly and cancer, particularly in patients with disease remission [300].

Establishing how elevated GH levels translate in to increased vascular morbidity and mortality remains elusive. One potential mechanism through which disturbances of the GH & IGF-I system increases vascular disease is via adverse effects on haemostasis and fibrinolysis, and subsequent clot formation.

5.2.3. Effects of acromegaly on thrombotic / fibrinolytic factors and platelet function

Abnormalities of coagulation and fibrinolysis have been considered to contribute to the increased risk of cardiovascular disease in patients with acromegaly. Elevated levels of plasma fibrinogen have been consistently reported by various research groups in patients with active acromegaly [568-575]. Fibrinogen has been previously recognised as an independent risk factor for cardiovascular disease and stroke [576]. A significant decrease in hyperfibrinogenaemia was found following treatment of acromegaly and biochemical remission of the condition [569, 570]. Similar fibrinogen levels were found in patients treated surgically and in those who achieved remission with somatostatin analogue therapy [569]. When compared with healthy controls, however, patients with biochemical disease control continued to have significantly elevated fibrinogen concentrations [569, 570, 577]. High fibrinogen levels in acromegalic patients have also been found in a case control study comparing a mixed group of patients with active and controlled acromegaly with patients with non-functioning pituitary adenomas [573]. A positive correlation between serum IGF-I and plasma fibrinogen levels has been reported [569, 572].

With regards to other markers of coagulation and fibrinolysis, data are scarce and often conflicting. Erem et al, in a cohort of 22 patients with active acromegaly, found elevated levels of AT-III, t-PA and PAI-1, along with reduced TFPI concentrations. The authors also reported a negative correlation between IGF-I and PAI-1 concentrations and between GH levels and TFPI [568]. Increased PAI-1 and t-PA levels have also been reported by Wildbrett et al, which in contrast to the previous study correlated positively with GH and IGF-I respectively [578]. The latter observations are supported by in vitro studies demonstrating increased PAI-1 production by hepatoma cells when grown in the presence of IGF-1 [579]. In

a third study, Landin-Wilhelmsen et al failed to demonstrate a significant difference in the PAI-1 levels between patients with active acromegaly and healthy controls [569]. Similarly, no difference in PAI-1, prothrombin fragments 1+2 and thrombin-antithrombin complex was found in a study by Sartorio et al, comparing 10 patients with acromegaly against 64 controls [575]. The same study also showed no difference in the values of t-PA between the acromegaly and control group, contrary to previous reports [575]. Lower levels of proteins C and S (which have an inhibitory effect on the coagulation cascade) have been found in patients with active acromegaly compared with healthy controls [572]. Additionally, patients with active acromegaly were found to have lower levels of protein S compared with patients with disease control [571]. However, when compared a mixed population of patients with active and controlled acromegaly against healthy volunteers higher protein S and no difference in protein C levels were shown [571].

In a recent study from Italy a more global assessment of coagulation and lysis was performed on a mixed population of 40 patients with acromegaly, including both treatment naïve patients, as well as patients following multi-modality therapy [580]. In addition to traditional markers of coagulation and fibrinolysis, the authors evaluated clot formation by thromboelastoplasty; platelet aggregation; and performed thrombin generation tests. The study showed higher levels of pro-coagulant factors (fibrinogen and factor VIII activity) in patients with acromegaly compared with controls, however no difference in proteins C and S, as well as PAI-1 and plasminogen activity was identified. Thromboelastometry showed faster clot formation and increased maximum clot firmness in patients with acromegaly, which in combination with the higher fibrinogen and factor VIII levels were indicative of increased thrombotic potential. In addition to the above, patients with acromegaly demonstrated increased endogenous thrombin generation compared with controls, with lesser inhibition of thrombin formation after the addition of thrombomodulin, suggesting a higher degree of resistance to the anticoagulant effects of thrombomodulin. However platelet aggregation was not different between patients and controls. Paradoxically, the authors reported an inverse correlation between GH/IGF-1 levels and thrombin generation, with lower endogenous

thrombin potential, higher antithrombin levels and a trend for lower Factor VIII activity amongst treatment naïve patients with acromegaly compared with those who have previously received treatment. However, this paradoxical finding could be attributed to the fact that a proportion of patients in the treated group continued to have active disease, as evident by the distribution of the GH values (median GH 0.72 mcg/L; interquartile range 0.51-4.26) [580].

In the few available studies on platelet function in patients with acromegaly, enhanced platelet function as evaluated by collagen/ADP and collagen-epinephrine-closure times was found in patients with active acromegaly compared with controls [572]. Additionally, increased mean platelet volume (a surrogate marker of platelet activation) has also been shown in case control study of 56 patients with acromegaly compared with 72 controls matched for age and sex [581]. The results from these studies also indicate increased thrombotic tendency as a results of enhanced platelet activation in patients with acromegaly; however these are in contrast with the findings of the recent Italian study in which no difference in platelet aggregation was found between patients with acromegaly and controls.

Regarding other non-traditional markers of cardiovascular risk, no difference in adiponectin levels (a peptide produced by the adipose tissue, which plays a role in inhibiting the metabolic processes that lead to insulin resistance, metabolic syndrome and atherosclerosis) was found in patients with active acromegaly compared with controls [582]. Similar homocysteine levels were found in a mixed population of 62 patients with active and controlled acromegaly compared with 36 controls [571]; however acromegalic patients with GH >2.5 mcg/L following an oral glucose tolerance test (OGTT) were found to have higher homocysteine levels compared with acromegalic patients with GH <2.5 post-OGTT in a small study of a total of 18 patients with acromegaly [583].

Overall, currently available data, although limited, suggest a degree of hypofibrinolysis and increased thrombogenic potential in acromegalic patients, with higher fibrinogen levels and platelet activity (Table 5.2). The haemostatic abnormalities seen in these patients appear to be at least partially reversible after biochemical disease control (both with medical and/or

surgical treatment [569, 577, 578]) and may contribute the increased cardiovascular risk associated with acromegaly.

A common caveat in most studies mentioned above is the focus on a single coagulation factor, which gives an incomplete picture of the thrombotic risk. A more comprehensive marker of thrombotic environment is fibrin network structure and susceptibility to lysis. This can be studied using a validated turbidimetric assay [584, 585]. The advantage of this technique is that it takes into account quantitative and qualitative changes in a large number of coagulation proteins, consequently translating the findings into alterations in fibrin clot properties. Recent work has shown that fibrin clot characteristics can predict adverse vascular outcomes in individuals sustaining a cardiac event, even after correction for a large number of clinical and biochemical vascular markers [586].

Establishing how elevated GH levels translate into increased vascular morbidity and mortality remains elusive. The hypothesis tested in this thesis is that one mechanism through which disturbances of the GH/IGF-I system increase vascular disease is by the induction of prothrombotic fibrin networks. The following cross-sectional study, evaluating properties of clot formation and lysis in a population of patients with acromegaly, was designed to test the above hypothesis.

Table 5.2. Summary of the current evidence on the main effects of acromegaly on several parameters of coagulation and fibrinolysis and their clinical significance.

Endocrine Disorder	**Main coagulation/fibrinolytic abnormalities**	**Overall effect on haemostasis**	**Clinical relevance**
Acromegaly	↑ Fibrinogen ↓ Protein C and S — t-PA, TFPI PAI-1 conflicting data Increased platelet activation	Possible mild hypercoagulability	Uncertain

5.3. SUBJECTS & METHODS

5.3.1. Study Outcomes

The primary outcome of the study was to evaluate clot structure properties in patients with acromegaly and to establish whether any differences exist in clot formation and clot lysis compared with controls, which in turn may contribute to the increased cardiovascular risk observed in acromegaly. Secondary outcomes include exploring the effect of disease activity on clot structure properties, aiming to assess whether normalisation of GH and IGF-1 reverses any potential abnormalities in the clotting and fibrinolysis processes. Other secondary outcomes include measuring key components of clot formation (fibrinogen) and lysis (PAI-1) and C reactive protein (CRP), a marker of vascular inflammation which has been associated with coronary artery disease [587], while assessing for conventional surrogates of cardiovascular risk (lipid profile, body composition, glucose profile, prevalence of metabolic comorbidities). This in-depth study on clot structure and function is novel and is expected to increase the understanding of the potential pathophysiological mechanism, through which GH excess increases the vascular morbidity of these individuals.

5.3.2. Participants' recruitment

This is a cross-sectional study, in which 40 consecutive patients with acromegaly were recruited. Acromegaly had been diagnosed in all patients prior to the recruitment to the study, by failure to suppress GH to <0.3 mcg/L, as measured by a two-site chemiluminescent immunometric human GH assay, during a 2-hour oral glucose tolerance test, with a 75g of oral glucose load. Patients were approached when attending for their clinic appointments and if they expressed an interest to the study, then their medical records were screened for the inclusion/exclusion criteria (listed below). Both patients with active disease and disease remission were approached, as well as patients with newly diagnosed acromegaly and patients who have previously received treatment for acromegaly.

In addition to the patient group, individually age and sex-matched healthy individuals were recruited from relatives of the patients and staff members of the Leeds Teaching Hospitals to provide control data. Patients' relatives were approached to take part to the study when accompanying patients to their clinic appointments. Hospital staff was recruited via advertisement material displayed in outpatient clinic areas, as well as circulated to all member of the Trust via the electronic Hospital's newsletter.

Written informed consent was obtained from all the participants. Ethical approval for the study was obtained from the North West - Greater Manchester West Research Ethics Committee (Reference ID: 15/NW/0400).

Based on the American Endocrine Society clinical practice guidelines 2014 [311] patients were divided into two groups: patients with disease remission (GH <1 mcg/L and IGF-1 within the age-specific reference range); and patients with active acromegaly (GH >1mcg/L and IGF-1 above the reference range) or dichotomous GH/IGF-1 results (GH <1mcg/L and IGF-1 above the reference range or GH >1mcg/L and IGF-1 within the reference range).

5.3.3. Inclusion/Exclusion criteria

Inclusion criteria

- Patients diagnosed with acromegaly at different stages of their disease course (i.e. newly diagnosed patients, as well as patients under surveillance following previous treatment) and at different degrees of disease activity (i.e. active disease, disease remission, discordant GH/IGF-1 results).

Exclusion criteria

- Individuals with a previous history of haematological disorder which predisposes to thrombotic or bleeding tendency

- Individuals already on treatment with antiplatelets (i.e. aspirin, clopidogrel, ticagrelol) or anticoagulants (i.e. warfarin, low-molecular weight heparin or novel oral anticoagulant medications)
- Patients with malignant disorders
- Individuals unable to provide informed consent

5.3.4. Cardiovascular risk assessment

5.3.4.i. Anthropometric measurements and assessment of metabolic parameters

Evaluation included measurement of weight; height; waist and hip circumference; skinfold thickness at bicep, tricep, infrascapular and suprailiac areas; body composition by bioelectrical impedance (Tanita TBF300MA, Middlesex, UK); blood pressure; pulse rate; and 12-lead ECG.

5.3.4.ii. Routine blood tests and sample collection

Measurements for the patient group include routine biochemistry (renal and liver function and calcium profile) as well as haematology tests (full blood count and clotting screen), full lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), fasting serum glucose and HbA1c. Patients were also tested for GH and IGF-1 to determine the disease status at the time of the study. In addition to the above, patients also had their anterior pituitary hormone profile tested including LH, FSH, oestradiol or testosterone (for female or male patients respectively), SHBG, TSH, free T4, prolactin and cortisol if the patient was not on glucocorticoid replacement due to adrenal insufficiency. Blood tests for controls included IGF-1, clotting screen, full lipid profile, fasting serum glucose and HbA1c.

Blood samples were obtained in the morning (8-10 am) following an overnight fast. The first 10mL of blood were used for clinical laboratory investigations (full lipid profile, fasting glucose, HbA1c), and additionally anterior pituitary hormone profile for the

patient group, including both random GH and IGF-1. A further 20mL blood sample was collected into a citrate tube, centrifuged at 4000 revolutions per minute and 1165 relative centrifugal force for 30 minutes, within two hours upon collection and the plasma stored at -80 ^{o}C until analysis. All blood samples were obtained without applying a tourniquet.

5.3.4.iii. Endothelial dysfunction

Endothelial dysfunction represents the earliest abnormality in the atherothrombotic process and it also has a crucial role in advanced stages of the disease. Estimation of urinary microalbumin excretion using albumin/creatinine ratio (ACR): microalbuminuria is an established cardiovascular risk factor and its presence correlates with endothelial dysfunction [588], and therefore analysis of ACR was used as an indirect method for the assessment of endothelial function. ACR was measured in a morning urine sample.

5.3.4.iv. Coagulation and Fibrinolysis factors

Plasma levels of fibrinogen and plasminogen activator inhibitor-1 (PAI-1) were determined using Clauss method and commercial ELISA (according to manufacturer's protocol) respectively.

5.3.5. Clot structure analysis

5.3.5.i. Turbidimetric analysis

This technique was used to analyse fibrin polymerisation characteristics in the clots formed ex vivo and to study fibrinolysis speed. Plasma samples were treated with thrombin and calcium using a microtiter plate spectrophotometer and changes in optical density were measured [589, 590]. Rates of fibrinolysis were analysed in the presence of tPA, both at the beginning of the clotting reaction and after formation of the mature clot. A number of clot structure parameters were studied (Figure 5.2) including:

(i) Maximum absorbance, which is a measure of fibrin network density and fibre thickness and equates to the maximum optical density of the blood clot. It has

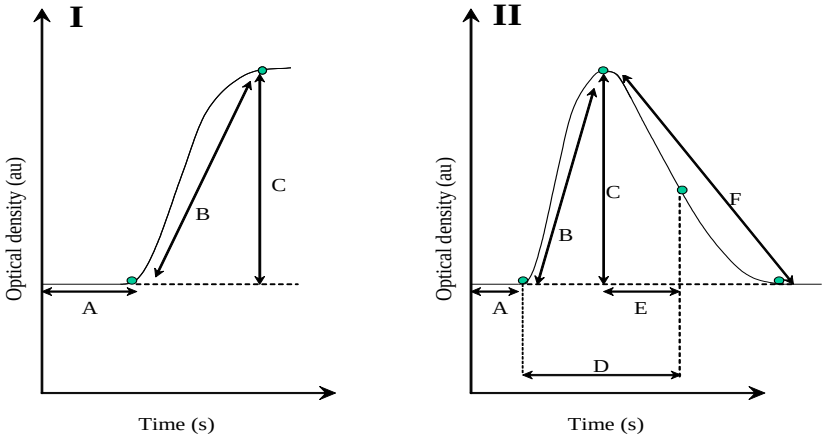
previously been shown that higher maximum absorbance is associated with increased cardiovascular risk [584, 589, 591].

(ii) Lag time, which is the time required from the start of the reaction after the addition of thrombin to the plasma until the beginning of clot formation. Shorter duration of lag time has been associated with increased thrombotic potential [584].

(iii) Lysis time, the time required for the clot to reach 50% lysis from the time of maximum absorbance (full clot formation). Longer lysis time indicates increased resistance to fibrinolytic mechanisms and has been associated with increased cardiovascular risk [585, 592].

(iv) Lysis area, which represents the area under the curve and is a complex measure, which combines clot formation time, clot density and lysis potential. Larger lysis area is associated with increased cardiovascular risk [589].

Figure 5.2. A summary of the different parameters analysed in turbidimetric assays. 'I' represents turbidity analysis, whereas 'II' demonstrates turbidity and lysis.



A: lag phase,
B: rate of clot formation,
C: maximum turbidity,
D: time from clot formation to 50% lysis
E: time from maximum clot formation to 50% lysis
F: rate of lysis

5.3.5.ii. Laser scanning confocal microscopy (LSCM)

Pooled plasma of all 40 patients with acromegaly was compared with pooled plasma of all the controls. Additionally, two pooled plasmas were produced; one of the patients with active acromegaly (n=22) and a second of the patients with disease remission (n=18). The pooled plasmas from each patient group were compared with controls, as well as with each other. Fibrin clots from these pooled samples were visualised using confocal microscopy.

Fibrin clots were created by diluting 7.5 μl from each pooled plasma with 20.4 μl of permeation buffer with the addition of Alexa 488-labelled fibrinogen at approximately 5% (0.105M) (Thermo Fisher Scientific/Life Technologies, Loughborough, UK) for 30 minutes at ambient temperature. Following incubation, activation mix consisting of 0.05U/mL human thrombin (Merck Chemicals Ltd, Nottingham, UK) and 5 mM/L CaCl2 in permeation buffer was added. The mixture was loaded to a 15-μl Ibidi (Applied Biophysics, Troy, NY) slide in duplicate to a well. The clots were visualised using a LSM880 microscope (Carl Zeiss, Welwyn Garden City, Hertfordshire, UK) using 40 x 1.4 oil objective lens. Three Z stacks of each clot were taken, with a range of 20.3 μm at intervals of 0.7 μm (total of 30 slices). The number of fibres per 100 μm was calculated in each stack using ImageJ® software. The average number from the three stacks was determined in each study group to represent the density of the clot fibrin network [589].

5.3.6. Laboratory assays

Fibrinogen was measured using the Clauss method [592], while PAI-1 and CRP were measured by commercial ELISA as per manufacturer's protocol [Thermo Fisher® Human PAI-1 Platinum ELISA BMS2033 and ab99995 – C Reactive Protein (CRP) Human ELISA Kit, respectively]. GH, IGF-1 and SHBG were measured using Siemens Immulite 2000 (GH calibrated against WHO NIBSC IS 98/574). Cortisol, prolactin and oestradiol were measured by radioimmunoassay on the Siemens Advia Centaur. LH, FSH, TSH and free T4 were measured by chemiluminescence using Advia Centaur, and testosterone was measured using

isotope-dilution liquid chromatography-tandem mass spectrometry. Total cholesterol, HDL cholesterol and triglycerides were measures by the ADVIA Chemistry Cholesterol Concentrated assay, ADVIA Chemistry Direct HDL Cholesterol and ADVIA Chemistry Triglycerides_2 Concentrated assay respectively, while LDL cholesterol was calculated using the Freidewald equation. Serum glucose was measured by an enzymatic assay based on the method by Slein, using hexokinase and glucose- 6- phosphate dehydrogenase enzymes. HbA1c was measured by the Tosoh G8 HPLC Analyzer, which utilises the Ion-Exchange method. All assays were performed in the routine clinical biochemistry laboratories within the Leeds Teaching Hospitals NHS Trust and have been regularly validated by internal quality control (IQC) and external quality assessment (EQA).

5.3.7. Statistical analysis

The study population has been calculated on the basis of two distinct endpoints representative of coagulation and body composition. (i) A total of 64 individuals are required to detect a 7% difference in turbidity analysis in response with a power of 80% at p=0.05, based on the standard deviation for the response variable is 10%. (ii) Previous studies employing a bioimpedence monitor to measure total body fat percentage have shown a SD of 10%. To detect a significant difference of 5% fat mass, at 5% significance with 80% power, requires 64 individuals (32 patients in each study group).

Descriptive data are presented as mean and standard deviation, or medial and interquartile range for parametric and non-parametric data respectively. Non-paired t-test for continuous variables and Mann-Whitney U-test (for variables which failed normality test) were used to assess the difference in the values between different comparison patient groups. The Chi-square or Fisher Exact test was used to compare proportions between the different study groups. Comparisons in the clot structure properties between patients and controls were also performed after adjusting for BMI, using univariate analysis of covariance test (ANCOVA).

Multiple linear regression analysis was also performed. The models used included lag time for clot formation, maximum clot optical density, time from maximum clot optical density to

50% lysis, lysis area, fibrinogen and PAI-1 as dependent values. For the patient group, independent values included patient's age at the time of the study; gender; BMI or fat mass or waist/hip ratio or summative skinfold thickness; GH at the time of the study or IGF-1 at the time of the study (expressed as % of upper limit of normal); use of GH/IGF-1 lowering medications at the time of the study; history of diabetes or impaired glucose tolerance; history of hypertension; dyslipidaemia; smoking status; duration of active disease and duration of disease remission. Fibrinogen was also included as an independent value in the regression models, which had lag time and maximum clot optical density as dependent values. PAI-1 was an independent value in the regression models in which lysis time and lysis area were tested as dependent values. For the control group, independent values included age; gender; BMI or fat mass or waist/hip ratio or summative skinfold thickness; smoking status; and levels of HbA1c, LDL and HDL cholesterol.

A P value of <0.05 was considered statistically significant. Statistical analysis was performed using the statistics software "SigmaPlot" (Systat Software Inc. London, UK).

5.4. RESULTS

A total of 92 patients with a history of acromegaly were screened for this study. Twenty-one patients were excluded as they were on treatment with antiplatelet or anticoagulant agents (4 patients due to previous deep vein thrombosis and/or pulmonary embolism; 4 patients due to history of ischaemic heart disease; 3 patients for thromboprophylaxis due to atrial fibrillation; 3 patients due to previous cerebrovascular disease/TIAs; 1 patient due to peripheral vascular disease; 1 patient due to metallic heart valve; 4 patients for primary prevention due other additional cardiovascular risk factors (i.e. diabetes, hypercholesterolaemia, hypertension); and 1 patient was on aspirin without a clear indication. Additionally, one patient was excluded due to myelodysplastic syndrome.

Thirty patients were excluded from the study for other reasons: 6 declined to take part to research as they were unable to take time off work; 5 patients had difficulties attending hospital appointments due to frailty and living far away; 5 patients had a poor attendance record at their clinic appointments; 2 patients had developed growth hormone deficiency following treatment for acromegaly (confirmed by dynamic pituitary test); 1 patient was undergoing chemotherapy for bowel cancer at the time of recruitment; 1 patient had cognitive impairment and was unable to provide informed consent; 1 patient was unable to speak or understand the English language and therefore was unable to provide informed consent; and finally, 9 patients declined to participate to the study without declaring any specific reason for their decision.

Eventually, 40 patients with acromegaly were recruited to the study.

5.4.1. Participants characteristics

5.4.1.i. Patient group

Forty patients with a diagnosis of acromegaly were recruited. Amongst them there were 21 male and 19 female patients, with a mean age of 53±13 years. With the exception of one patient who was diagnosed with a GHRH-secreting bronchial carcinoid causing acromegaly, the remaining cases of acromegaly were due to GH-secreting pituitary adenomas. Based on

the American Endocrine Society clinical practice guidelines 2014 [311], 27.5% of patients (n=11) had active disease (GH >1mcg/L and IGF-1 above the age-specific reference range); 27.5% (n=11) had dichotomous GH/IGF-1 results (GH <1mcg/L and IGF-1 above the reference range or GH >1mcg/L and IGF-1 within the reference range); and 45% of patients (n=18) were in remission at the time of the study (GH <1mcg/L and IGF-1 within the age-specific reference range). For the entire patient cohort the median GH and IGF-1 were 0.85 (0.375-2.325) mcg/L and 97.9 (64.5-140.6)% of the upper limit of normal (ULN) respectively.

The mean age at diagnosis of acromegaly was 40.8±12.3 years and the estimated age at the onset of symptoms of acromegaly was 33.5±12.2 years, based on patients' reports. The mean duration of active disease (estimated from the time on onset of symptoms until the time disease remission was achieved) was 14±10.2 years, while the mean duration of disease remission was calculated at 5.0±6.0 years. Thirst-five patients (87.5%) had trans-sphenoidal surgery, while 5 patients did not have any surgical intervention to their pituitary gland (one patient had apoplexy following the diagnosis of acromegaly; three patients were recently diagnosed with acromegaly and were recruited to the study prior to having pituitary surgery; and two patients were managed medically only). Of the patients who underwent trans-sphenoidal surgery, 24 had surgery once, 10 had surgery twice, while one patient required surgery three times. Sixteen patients (40%) had radiotherapy to the pituitary gland, which in all cases was an adjuvant therapy to pituitary surgery. External beam radiotherapy 45 Gy in 25 fractions was delivered in 12 cases (75%), while in the remaining 4 cases (25%) the patients had gamma-knife radiotherapy to the GH-secreting pituitary adenoma.

The majority of patients (n=32, 80%) had been on medical therapy during the course of their disease for a mean of 8.5±7.5 years. Thirty patients were treated with somatostatin analogues (either Octreotide LAR or Lanreotide) and eight patients received treatment with dopamine agonists (cabergoline or bromocriptine), in conjuction with somatostatin analogues in six cases and as monotherapy in the remaining two. No patient in this study had been treated with

the GH receptor antagonist Pegvisomant. Overall, 21 patients (52.5%) were on medical therapy at the time of the study.

Following multi-modality treatment for acromegaly, 18 patients (45%) had developed hypopituitarism; 17 patients (42.5%) had LH/FSH deficiency; 13 patients (32.5%) had ACTH deficiency; 10 patients (25%) had TSH deficiency; and 3 patients (7.5%) had diabetes insipidus (ADH deficiency). However, all the above patients had been on long-term (for at least 3 months prior to the study) appropriate hormone replacement therapy with the exception of post-menopausal women with gonadotrophin deficiency, in which oestrogen replacement therapy was not clinically indicated. For patients with ACTH deficiency, the total daily dose range of hydrocortisone used as a replacement was 15-25mg.

With regards to the initial tumour size, 17 patients (42.5%) were found to have a macroadenoma (>10mm), 5 patients (12.5%) had a mesoadenoma (5-10mm), 4 patients (10%) had a microadenoma (<5mm), in one case the patient had pituitary hyperplasia rather than adenoma secondary to ectopic GHRH secretion from a bronchial carcinoid causing pituitary stimulation, while in 13 cases (32.5%) no data could be found regarding initial pituitary tumour size. Data are summarised in Table 5.3.

5.4.1.ii. Control Group

Forty healthy volunteers matched for age and sex with patients were recruited. Mean age of controls was 53.2±12.5 years (p=0.917). Four controls (10%) were previously diagnosed with hypertension and were established on treatment, with reasonable blood pressure control (BP range at the time of the study 129/87 – 143/89 mmHg); one control was on atorvastatin for primary prevention; one had primary hypothyroidism and had been on a stable dose of levothyroxine for a number of years; one was on a progesterone implant; and another control was of female hormone replacement (HRT) for the management of menopausal symptoms. None of the controls had a previous cardiovascular or cerebrovascular event or diagnosis of diabetes.

Table 5.3. Summary of the acromegaly-related past medical history for the patient group (N=40)

	Number of patients
Current disease status	
• In remission	18 (42.5%)
• Active	11 (27.5%)
• Dichotomous GH/IGF-1 results	11 (27.5%)
Current GH (mcg/L)	0.85 (0.375-2.325)
Current IGF-1 (% ULN)	97.9 (64.5-140.6)
Mean age at diagnosis of acromegaly (years)	40.8±12.3
Estimated age at onset of symptoms (years)	33.5±12.2
Duration of active disease (years)	14±10.2
Duration of disease remission (years)	5.0±6.0
GH at diagnosis (mcg/L)	21±21.3
IGF-1 at diagnosis (% ULN)	288.8±121.8
Trans-sphenoidal surgery	35 (87.5%)
Cranial radiotherapy	16 (40%)
Medical therapy	32 (80%)
Hypopituitarism (any)	18 (45%)
• LH/FSH deficiency	17 (42.5%)
• ACTH deficiency	13 (32.5%)
• TSH deficiency	10 (25%)
• ADH deficiency	3 (7.5%)

5.4.2. Traditional Markers of Cardiovascular Risk

5.4.2.i. Metabolic Profile / Body composition

Compared with controls, patients had higher BMI (30±5.5 vs. 26.7±4.1 kg/m^2, p=0.003), waist/hip ratio (0.91±0.08 vs. 0.87±0.08, p=0.045) and total fat mass (29.8±10 vs. 23.4±10 kg, p=0.003). No difference was found in lean body mass (LBM) and total body weight (TBW) between patients and controls. With regards to skinfold thickness (SKF), a significant difference was found only in the infrascapular area, with higher SKF in patients (23.2±8.3 vs.19.7±7.8 mm, p=0.045); however no difference was seen in the suprailiac, biceps, triceps and summative SKF between patients and controls.

5.4.2.ii. Metabolic Comorbidities

Amongst patients with acromegaly, 3 had diabetes (one patient with insulin-dependent diabetes, diagnosed after having presented with diabetic ketoacidosis simultaneously with his clinical diagnosis of acromegaly; one patient with Type 2 diabetes; and one patient with diet controlled diabetes) and 2 patients had impaired glucose tolerance (IGT) but not diabetes. In contrast, none of the controls had a diagnosis of diabetes or IGT prior to the study (p=0.055, Fisher Exact test for the combined prevalence of diabetes and IGT). Thirteen patients (32.5%) had hypertension (9 of them were on treatment) compared with four controls (p=0.03, Chi-square test). Eight patients (20%) had a diagnosis of hypercholesterolaemia prior to the study (however only 4 were on treatment with statins), compared with one control individual (2.5%), who had dyslipidaemia and was on a statin (p=0.03, Fisher Exact test). Twenty-one patients (52.5%) had never smoked; fourteen patients (35%) were ex-smokers; while only five patients (12.5%) were actively smoking at the time of the study. There was no difference in the proportion of smokers/non-smokers between patients and controls. Amongst the patients with a smoking history, the

estimated mean number of pack years was 21.5±16.2. None of the patients or controls had previously sustained any cardiovascular or cerebrovascular event.

5.4.2.iii. Laboratory Results

Patients had higher triglyceride levels compared with controls [median 1.35mmol/L (25-75% IQR 0.9-1.7) vs. 1.00mmol/L (25-75% IQR 0.8-1.3); p=0.018]. However, there was no difference in the remaining parameters of lipid profile between patients and controls. Additionally, no difference was observed in the fasting serum glucose levels and HbA1c between the two groups. Values of urine albumin/creatinine ratio (ACR) were also similar in patients and controls [median ACR 0.425 mg/mmol (25-75% IQR 0.3-0.88) vs. 0.5 mg/mmol (25-75% IQR 0.29-1.3), p=0.88, respectively].

Regarding traditional markers of clotting, no difference in the INR, PT was observed between patients and controls [median INR 0.9 (25-75% IQR 0.9-1.0) vs. 0.9 (25-75% IQR 0.9-1.0), p=0.141; median PT 11 sec (25-75% IQR 10-11) vs. 11 sec (25-75% IQR 10-11), p=0.852, respectively]. However, a more prolonged aPPT was found in the patient group (mean aPTT 33.1±3.3 sec vs. 31.6±3.2 sec, p=0.046). One patient particularly had an elevated aPTT at 39.4 seconds; he was also found to have abnormal liver function at the time of the study with an ALT of 294 iu/L (reference range <40 iu/L). The patient was not known to have a liver disorder and repeat tests of liver function and clotting screen two months later showed normal ALT at 31 iu/L and normal clotting screen with an aPTT of 28.3 seconds. After excluding this patient, the mean aPTT value for the patient group was 32.9±3.2 sec and the difference was no longer significant when compared with the mean aPTT value for controls (p=0.069). A summary of the above results can be found in Table 5.4.

Table 5.4. Summary of the metabolic profile for patients (N=40) and controls (N=40).

	Patients (N=40)	**Controls** (N=40)	**p-value**
Age (years)	53±13	53.2±12.5	0.917
Male/Female	21/19	21/19	1.00
Body Composition			
BMI (kg/m^2)	30±5.5	26.7±4.1	0.003
Waist hip ratio	0.91±0.08	0.87±0.08	0.045
Total Fat Mass (kg)	29.8±10	23.4±10	0.003
Total LBM (kg)	58.6±13.8	53.8±12.2	0.09
TBW (kg)	42.9±10.1	39.4±9.0	0.09
SKF			
Biceps (mm)	11.3±5.6	12.4±5.2	0.36
Triceps (mm)	12.9±7.7	14.8±10.2	0.55
Infrascapular (mm)	23.2±8.3	19.7±7.8	0.045
Suprailiac (mm)	15.3±6.7	14.4±6.5	0.57
Summative (mm)	62.6±21.9	61.3±22.7	0.73
Cardiovascular Risk Factors			
Total Cholesterol (mmol/L)	5.15 (IQR 4.4-5.75)	5.42 (IQR 4.6-6.0)	0.33
LDL (mmol/L)	2.9 (IQR 2.2-3.9)	3.2 (IQR 2.6-3.8)	0.1
HDL (mmol/L)	1.5 (IQR 1.2-2.1)	1.7 (IQR 1.4-2.1)	0.34
Triglycerides (mmol/L)	1.35 (IQR 0.9-1.7)	1.0 (IQR 0.8-1.3)	0.018
Fasting glucose (mmol/L)	4.85 (IQR 4.4-5.3)	4.6 (IQR 4.3-5.0)	0.23
HbA1c (mmol/mol)	37 (IQR 34-41)	37 (IQR 33-40)	0.19
Diabetes/IGT	5	0	0.055
Hypertension	13	4	0.03
Dyslipidaemia	8	1	0.03
Smokers/Ex-smokers/Non-smokers	5/14/21	2/9/21	0.47

5.4.3. Clot Structure Analysis

Patients had raised clot maximum absorbance (a measure of clot density), compared with controls (0.38±0.13 vs. 0.32±0.08 arbitrary units, p=0.02). No difference in the lag time for clot formation was observed between patients and controls (540.2±75 sec vs. 530.3±80.5 sec, respectively; p=0.57).

Regarding fibrinolysis, no statistical difference in clot lysis times was found between the two groups at all lysis stages [median times from maximum clot optical density to 25%, 50% and 75% lysis were respectively: 8.7 (7.6-13.8) min vs. 8.6 (7.3-11.2) min, p=0.44; 18.4 (15.1-38.3) min vs. 17.9 (14.4-30.3) min, p=0.56; and 36.3 (23.8-52.3) min vs. 34.1 (25.6-45.6) min, p=0.7]. However, there was a trend towards greater lysis area in the patient group compared with controls [median 634 (452-905) vs. 501 (429-784) arbitrary units, respectively, p=0.08]. Results are also presented in Table 5.5. Figure 5.3 shows the changes in the clot optical density during clot formation and clot lysis collectively for the patient group in comparison with controls.

5.4.4. Coagulation proteins and CRP plasma levels

Patients with acromegaly had significantly higher median fibrinogen levels compared with controls [3.1 (2.6-4.1) vs. 2.6 (2.5-2.7) mg/ml, respectively, p<0.001)], which is in-keeping with the higher clot MA observed in the patient group. Median PAI-1 levels were similar in the two study groups [3.7 (0.95-9.86) vs. 4.0 (1.3-8.1) ng/ml, for patients and controls respectively, p=0.57], which is also consistent with the lysis data. No difference in median CRP levels was observed between patients and controls [2.91 (0.1-13.0) vs. 3.42 (1.0-11.4) mg/L, respectively; p=0.46]. Results are also shown in Table 5.5.

Figure 5.3. Changes in the mean clot optical density with time (sec) in patients with acromegaly (N=40, blue line) and healthy controls (N=40, red line). The y axis represents clot absorbance (a measure of clot optical density), while the x axis represents time (in seconds) from the start of the turbidimetric analysis. Maximum clot absorbance was higher in patients with acromegaly suggestive of increased thrombotic potential with more compact clots in the patient group. Lysis area (area under the curve) was significantly higher in patients compared with controls, suggesting that both clot formation and fibrinolysis are impaired in patients with acromegaly. Measurements on clot optical density were performed on individual serum samples, rather than pooled samples. The average clot optical density was then calculated for each study group and then presented in the graphs.

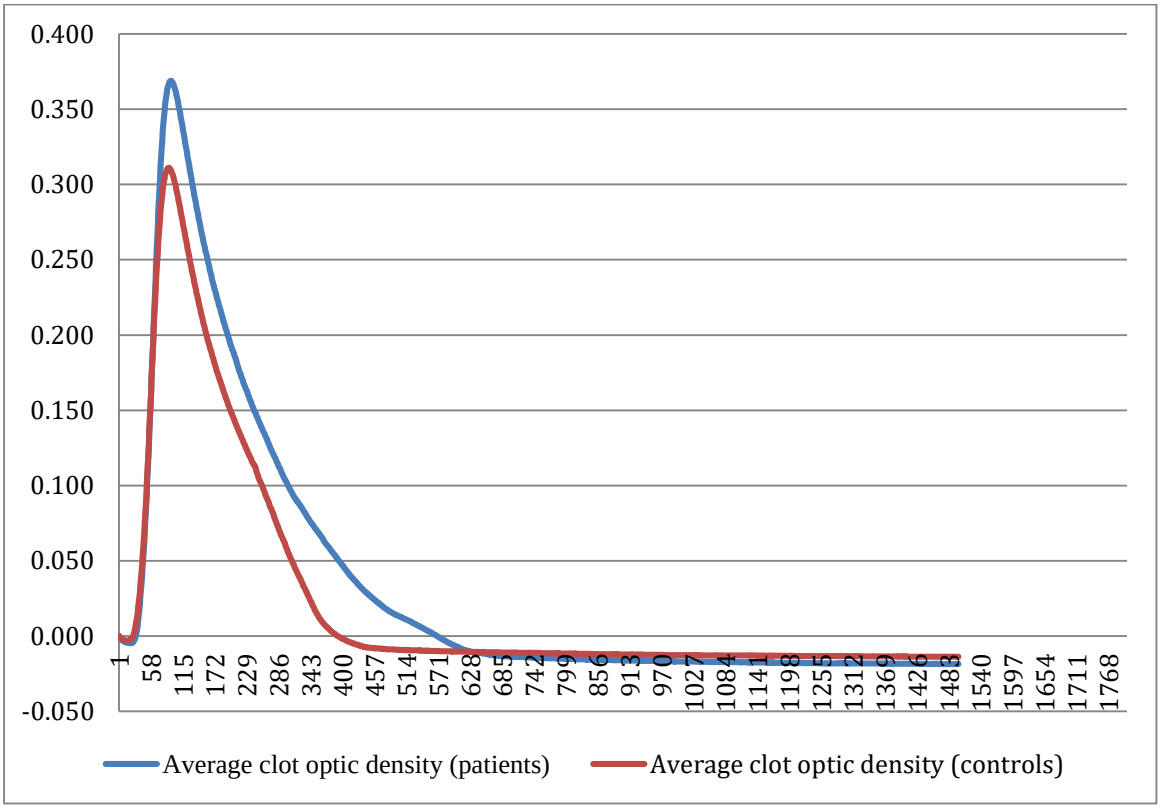


Table 5.5. Comparison in clot structure properties between patients with acromegaly (N=40) and controls (N=40)

	Patients (N=40)	**Controls** (N=40)	**P-value**
Lag time (sec)	540.1±75	530.3±80.5	0.57
Maximum optical density (arbitrary units)	0.38±0.13	0.32±0.08	**0.02**
50% Lysis time (min)	29±22.2	23.2±11.8	0.15
Lysis Area (arbitrary units)	844.4±672.7	569.7±246.7	**0.018**
Fibrinogen (mg/ml)	3.59±1.56	2.58±0.37	**<0.001**
PAI-1 (ng/ml)	8.9±11.6	5.0±4.6	0.0535

5.4.5. Correlations

5.4.5.i. Patient group

Multiple linear regression analysis with adjustment for confounding factors was also performed as described in methodology. Lag time for the start of clot formation was negatively associated with fibrinogen levels (co-efficient -17.8, p=0.012), current GH values (co-efficient -11.5, p=0.021), and smoking (co-efficient -32.5, p=0.036).

Maximum clot optical density was positively associated with fibrinogen levels (co-efficient 0.06, p<0.001), BMI (co-efficient 0.008, p=0.044), total fat mass (co-efficient 0.004, p=0.041) and summative skinfold thickness (co-efficient 0.002, p=0.048). No associations were found between maximum clot optical density and acromegaly-specific factors such as GH, IGF-1, duration of active disease and disease remission.

Lysis time (time from maximum optical density to 50% lysis) was positively correlated with PAI-1 levels (coefficient 1.1, p<0.001), diabetes (co-efficient 10.9, p=0.03), and summative skinfold thickness (co-efficient 0.28, p=0.043), with a trend for BMI (co-efficient 1.08, p=0.07). Acromegaly-specific parameters were not found to be predictive of lysis time.

Lysis area was positively correlated with PAI-1 levels (coefficient 14.7, p<0.001); older patient's age (coefficient 10.1, p<0.001); and summative skinfold thickness (coefficient 2.8, p=0.012). There was also a negative correlation between lysis area and duration of remission of acromegaly (coefficient -11.8, p=0.015).

A positive correlation was found between serum fibrinogen and duration of active disease (co-efficient 0.06, p=0.034) and smoking (co-efficient 0.9, p=0.038), whereas PAI-1 was positively associated with BMI (coefficient 0.87, p=0.039). A summary of the results of the multiple linear regression analysis after adjustment for confounding factors can be found in Table 5.6.

5.4.5.ii. Control group

A negative correlation between lag time and WHR was found (co-efficient -549.4, p=0.015). Clot MA was positively correlated with fibrinogen (co-efficient 0.167, p<0.001) and lysis time and lysis area with PAI-1 levels (co-efficient 1.423, p=0.01 and 19.39, p=0.02 respectively). Total fat mass, WHR and summative skinfold thickness were positively correlated with fibrinogen (coefficient 0.013, p=0.03; 2.074, p=0.025; and 0.01, p=0.005 respectively), but not directly with clot MA. Finally, a positive correlation was found between PAI-1 and HbA1c (co-efficient 0.43, p=0.02); PAI-1 and WHR (co-efficient 32.5, p=0.006); and PAI-1 and total fat mass (co-efficient 0.17, p=0.038). A summary of these results can be found in Table 5.7.

Table 5.6. Summary of correlations based on multiple linear regression analysis after adjusting for confounding factors (patient group). A p-value of <0.05 was considered statistically significant. NS refers to statistically non-significant results. N/A: non-applicable**.**

PATIENTS																	
	Patient's age	**Gender (1=male, 2=female)**	**BMI**	**Total fat mass**	**Summative skinfold thickness**	**WHR**	**Current GH**	**Current IGF-1**	**Diabetes (0=no diabetes, 1=pre-diabetes, 2=diabetes)**	**Hypertension (0=no, 1=yes)**	**Dyslipidaemia (0=no, 1=yes)**	**Smoking (0=no, 1=ex-smoker, 2=current smoker)**	**Duration of active disease**	**Duration of disease remission**	**Current medical therapy (0=no, 1=yes)**	**Fibrinogen**	**PAI-1**
Lag time	Coefficient 1.7, p=0.035	NS	NS	NS	NS	NS	Coefficient -11.5, p=0.021	NS	NS	Coefficient -39.7, p=0.056	NS	Coefficient -32.5, p=0.036	NS	NS	NS	Coefficient -17.8, p=0.012	N/A
Max OD	NS	NS	Coefficient 0.008, p=0.044	Coefficient 0.004, p=0.041	Coefficient 0.002, p=0.048	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	Coefficient 0.06, p<0.001	Coefficient 0.004, p=0.02
50% Lysis Time	NS	NS	Coefficient 1.09, p=0.07	NS	Coefficient 0.28, p=0.04	NS	NS	NS	Coefficient 10.9, p=0.03	NS	NS	NS	NS	NS	NS	N/A	Coefficient 1.1, p<0.001
Lysis Area	Coefficient 10.1, p<0.001	NS	NS	NS	Coefficient 2.8, p=0.012	NS	NS	NS	NS	NS	NS	NS	NS	Coefficient -11.8, p=0.015	NS	NS	Coefficient 14.7, p<0.001
Fibrinogen	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	Coefficient 0.9, p=0.038	Coefficient 0.06, p=0.034	NS	NS	N/A	N/A
PAI-1	NS	NS	Coefficient 0.868, p=0.039	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	N/A	N/A

Table 5.7. Summary of correlations based on multiple lineal regression analysis after adjusting for confounding factors (control group). A p-value of <0.05 was considered statistically significant. NS refers to statistically non-significant results. N/A: non-applicable.

Controls															
	Age	**Gender (1=male, 2=female)**	**BMI**	**Total fat mass**	**Summative skinfold thickness**	**WHR**	**Total cholesterol**	**LDL**	**HDL**	**Triglycerides**	**Hypertension (0=no, 1=yes)**	**Smoking (0=no, 1=ex-smoker, 2=current smoker)**	**HbA1c**	**Fibrino-gen**	**PAI-1**
Lag time	NS	NS	NS	NS	Coefficient 1.64, p=0.06	Coefficient -549.4, p=0.015	NS	NS	NS	NS	NS	Coefficient -73.7, p=0.01	NS	Coefficient -129.5, p=0.02	NS
Max OD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	Coefficient 0.167, p<0.001	NS
50% Lysis Time	NS	NS	NS	NS	NS	NS	NS	NS	NS	Coefficient 13.4, p=0.05	Coefficient -29.3, p=0.044	NS	NS	NS	Coefficient 1.423, p=0.01
Lysis Area	NS	NS	NS	NS	NS	NS	NS	NS	NS	Coefficient 13.4, p=0.05	Coefficient -29.3, p=0.044	NS	NS	NS	Coefficient 19.39, p=0.02
Fibrinogen	Coefficient 0.01, p=0.04	Coefficient 0.49, p=0.002	NS	Coefficient 0.013, p=0.03	Coefficient 0.01, p=0.005	Coefficient 2.074, p=0.025	NS	NS	Coefficient -0.38, p=0.02	NS	NS	NS	NS	N/A	N/A
PAI-1	NS	NS	NS	Coefficient 0.17, p=0.038	NS	Coefficeint 32.5, p=0.006	NS	NS	NS	NS	NS	NS	Coefficient 0.43, p=0.02	N/A	N/A

5.4.6. Assessing the impact of disease activity on clot structure

5.4.6.i. *Patients' characteristics*

A further sub-analysis was performed dividing the patient groups into two subgroups: patients with disease remission (GH<1mcg/L and IGF-1 within normal limits) and patients with active acromegaly including the remaining patients in whom the remission criteria did not apply. Therefore the active disease group included patients with discordant GH/IGF-1 results as well.

Twenty-two patients with active acromegaly (Group 1; mean age 51±13 years) and 18 with disease remission (Group 2; mean age 55±13 years) were assessed. Median GH and IGF-1 values were significantly higher in the group with active acromegaly as expected [GH: 2.6 (0.8-3.5) mcg/L vs. 0.3 (0.1-0.68) mcg/L, $p<0.001$ and IGF-1: 132 (107-212)% vs. 73 (58-94)% of upper limit of normal, $p<0.001$ respectively]. Median duration of disease remission in Group 2 was 8.25 (4.1-13.9) years. Table 5.8 summarises patients' clinical characteristics and acromegaly-related medical history.

Compared with patients with active acromegaly, patients in remission had higher LDL and triglyceride levels. There was no difference in the two groups with regards to age, gender distribution, BMI, fat mass (as measured by bioimpedance), skinfold thickness, total cholesterol and HbA1c. Patients with active disease had significantly higher BMI, LDL cholesterol and prevalence of diabetes/IGT compared with controls. Patients with disease remission also demonstrated a less favourable metabolic and cardiovascular risk profile compared with controls, due to higher BMI, waist/hip ratio (WHR), fat mass, triglycerides and prevalence of hypertension and dyslipidaemia. Results are summarised in Table 5.9.

Table 5.8. Summary of the acromegaly-related past medical history (biochemical results, disease status at the time of the study, previous therapeutic interventions and pituitary-related outcomes) for patients with active disease (n=22) and disease remission (n=18).

	Patients with active acromegaly (n=22)	**Patients with disease remission** (n=18)	**p-value**
Age (years)	51±13	55.3±13	0.3
Male/Female	13/9	8/10	0.545
Current GH (mcg/L)	2.6 (0.8-3.5)	0.3 (0.1-0.68)	**<0.001**
Current IGF-1 (% ULN)	131.7 (106.9-212.2)	72.5 (58.3-94.0)	**<0.001**
Mean age at diagnosis of acromegaly (years)	40.6±12.8	40.9±12.0	0.93
Estimated age at onset of symptoms (years)	33.7±12.5	33.2±12.2	0.9
Duration of active disease (years)	11.8 (8.25-23.5)	6.0 (5.0-16.5)	0.11
Duration of disease remission (years)	0 (0-2.0)	8.25 (4.1-13.9)	**<0.001**
GH at diagnosis (mcg/L)	19.4 (5.9-33.3)	8.55 (4.1-22.5)	0.34
IGF-1 at diagnosis (% ULN)	307 (158.7-413.5)	275.7 (185.4-368.4)	0.85
Trans-sphenoidal surgery	19 (86.4%)	16 (88.9%)	1.00
Cranial radiotherapy	13 (59.1%)	7 (38.9%)	0.42
Medical therapy	14 (63.6%)	8 (44.4%)	0.37
Hypopituitarism			
• LH/FSH deficiency	6 (27.3%)	7 (38.9%)	0.66
• ACTH deficiency	9 (40.9%)	8 (44.4%)	0.92
• TSH deficiency	5 (22.7%)	5 (27.8%)	0.7
• ADH deficiency	0 (0%)	3 (16.7%)	0.08

Table 5.9. Body composition, lipid and glucose profile and prevalence of cardiovascular risk factors in patients with active acromegaly (n=22), patients in remission (n=18) and control subjects (n=40). BMI: body mass index; LBM: lean body mass; SKF: skinfold thickness; TBW: total body weight; WHR: waist-hip ratio.

	Active disease	**Disease remission**	**p-value**	**Active disease**	**Controls**	**p-value**	**Disease remission**	**Controls**	**p-value**
Age (years)	51±13	55.3±13	0.3	51±13	53.2±12.5	0.5	55.3±13	53.2±12.5	0.56
Male/Female	13/9	8/10	0.545	13/9	21/19	0.82	8/10	21/19	0.78
BMI (kg/m^2)	29.4±5.3	30.8±5.7	0.45	29.4±5.3	26.7±4.1	**0.03**	30.8±5.7	26.7±4.1	**0.003**
WHR	0.9±0.08	0.92±0.08	0.43	0.9±0.08	0.87±0.08	0.2	0.92±0.08	0.87±0.08	**0.044**
Total Fat Mass (kg)	28.3±11	31.6±8.8	0.3	28.3±11	23.4±10	0.08	31.6±8.8	23.4±10	**0.004**
Total LBM (kg)	58.3±12.3	58.9±15.7	0.9	58.3±12.3	53.8±12.2	0.17	58.9±15.7	53.8±12.2	0.18
TBW (kg)	42.7±9	43.2±11.5	0.9	42.7±9	39.4±9.0	0.17	43.2±11.5	39.4±9.0	0.18
Summative SKF (mm)	59±21.5	66.9±22.2	0.26	59±21.5	61.3±22.7	0.71	66.9±22.2	61.3±22.7	0.38
Total Cholesterol (mmol/L)	5.0±1.0	5.8±1.5	0.06	5.0±1.0	5.4±0.9	0.09	5.8±1.5	5.4±0.9	0.3
LDL (mmol/L)	2.7±0.8	3.45±1.3	**0.03**	2.7±0.8	3.2±0.75	**0.017**	3.45±1.3	3.2±0.75	0.4
HDL (mmol/L)	1.7±0.6	1.6±0.5	0.6	1.7±0.6	1.7±0.45	0.74	1.6±0.5	1.7±0.45	0.3
Triglycerides (mmol/L)	1.3±0.9	1.7±0.8	**0.04**	1.3±0.9	1.1±0.5	0.19	1.7±0.8	1.1±0.5	**0.001**
Fasting glucose (mmol/L)	4.9±0.9	4.9±0.65	0.96	4.9±0.9	4.7±0.4	0.17	4.9±0.65	4.7±0.4	0.1
HbA1c (mmol/mol)	41.2±12.9	39.7±8.9	0.67	41.2±12.9	37±4.5	0.065	39.7±8.9	37±4.5	0.12
Diabetes/Impaired glucose tolerance	3	2	1.0	3	0	**0.04**	2	0	0.09
Hypertension	6	7	0.66	6	4	0.14	7	4	**0.025**
Dyslipidaemia	3	5	0.43	3	1	0.12	5	1	**0.009**
Smokers/Ex-smokers/Non-smokers	4/6/12	0/8/9	0.13	4/6/12	2/9/21	0.38	0/8/9	2/9/21	0.29

5.4.6.ii. *Clot structure analysis*

Patients with active acromegaly had shorter lag time for the initiation of clot formation (514±72 vs. 571±68 sec, p=0.02), however maximum clot optical density was similar in the two groups (Group 1: 0.40±0.12 vs. Group 2: 0.35±0.14 arbitrary units, p=0.26). Additionally, no statistical difference was found either in the lysis times [Group 1: median 18.1 (15.9-25.1) min; Group 2: 24.5 (13.9-58.6) min; p=0.49] or in the lysis areas between the two groups [Group 1: median lysis area 634 (484-848) AU; Group 2: 614 (427-1422) AU, p=0.86].

Following adjustment for BMI, no significant change to the results occurred. Patients with active acromegaly had shorter lag time compared with those in remission (515.6±15.1 vs. 570.2±16.8 sec, p=0.02), however clot MA was similar in the two groups (Group 1: 0.41±0.03 arbitrary units (AU); Group 2: 0.35±0.03 AU, p=0.18). Additionally, no statistical difference was found either in 50% lysis time [Group 1: 25.5±4.3 min; Group 2: 33.2±4.8 min; p=0.24] or lysis areas [Group 1: 849.7±130.9 AU; Group 2: 837.9±144.8 AU, p=0.95]. Results are summarised in Table 5.10.

When patients from each subgroup were compared with their respective controls, patients with active disease had significantly higher maximum clot optical density (0.4±0.12 vs. 0.32±0.08 AU, p=0.004). No difference was found in the lag time for clot formation (514.7±72 vs. 542.7±65.8 sec, p=0.18); lysis time [median 50% lysis time 18.1 (15.9-25.1) min for patients with active acromegaly vs. 17.9 (14.4-30.3) min for controls, p=0.96] and lysis area (median lysis area 634 (484-848) AU for patients with active acromegaly vs. 501 (429-784) AU for controls, p=0.14). In contrast, there was no difference in any of the clot formation and lysis parameters between patients with disease remission and controls.

Following adjustment for BMI, patients with active disease continued to have significantly higher clot MA (0.39±0.02 vs. 0.32±0.01 AU, p=0.03). There was a trend towards longer lysis time and larger lysis area for patients with disease remission compared with controls (50% lysis time 33.5±4.3 vs. 23.6±2.8 min, p=0.066 and lysis area 834.5±99.8 vs.

590.1±65.9, p=0.053), although the difference did not reach statistical significance (Table 5.10).

Figure 5.4 illustrates the changes in the clot optical density during clot formation and lysis in patients with active acromegaly, patients with disease remission and the control group.

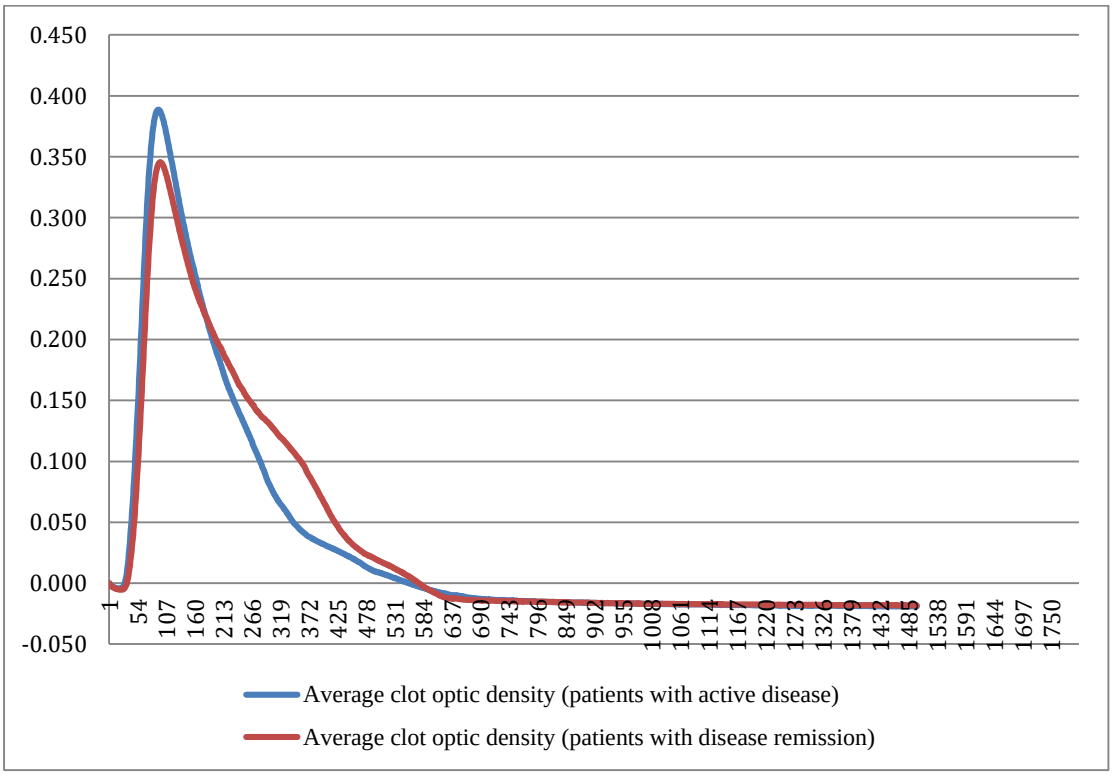
5.4.6.iii. *Coagulation proteins and CRP plasma levels*

There was a trend towards higher fibrinogen levels in patients with active acromegaly compared with patients in remission [Group 1: median 3.45 (2.7-4.4) mg/ml; Group 2: 2.8 (2.4-3.5) mg/ml, p=0.08]; however, PAI-1 levels were similar in the two patient groups [Group 1: median 3.7 (0.6-9.5) ng/ml; 5.5 (1.9-21.0) ng/ml, p=0.25]. No difference in CRP levels was detected. Following adjustment for BMI no difference in fibrinogen, PAI-1 and CRP was observed between patients with active acromegaly and those in remission (Table 5.10).

Patients with active disease had significantly higher fibrinogen concentrations compared with controls [median 3.45 (2.7-4.4) vs. 2.6 (2.5-2.7) mg/ml, respectively, p<0.001)], which is in-keeping with the higher clot MA observed in the patient group. PAI-1 levels were similar in the two study groups, which is also consistent with the lysis data. No difference in plasma CRP was observed. No difference in fibrinogen, PAI-1 and CRP was observed between patients with disease remission and controls.

Following adjustment for BMI, both patients with active acromegaly and disease remission had significantly higher fibrinogen levels compared with controls; however the difference was greater for patients with active disease (patients with active acromegaly vs. controls: 3.8±0.2 vs. 2.6±0.2 mg/ml, respectively, p<0.001; patients in remission vs. controls: 3.25±0.2 vs. 2.6±0.2 mg/ml, respectively, p=0.02). No difference in PAI-1 and CRP was observed between patients with active acromegaly and controls, as well as patients with disease remission and controls (Table 5.10).

Figure 5.4. (i) Changes in the mean clot optical density with time (sec) in patients with active acromegaly (n=22, blue line) and in patients with disease control (n=18, red line). The y axis represents clot absorbance (a measure of clot optical density), while the x axis represents time (in seconds) from the start of the turbidimetric analysis. No significant difference in the parameters of clot formation and lysis was observed. (ii) Changes in the mean clot optical density with time (sec) in patients with active acromegaly (n=22, blue line) and their respective controls (n=22, red line). Patients with active disease had significantly higher maximum clot absorbance at the end of clot formation, suggesting higher clot density in the active disease group. (iii) Changes in the mean clot optical density with time (sec) in patients with disease remission (n=18, blue line) and their respective controls (n=18, red line). No significant difference in the parameters of clot formation and lysis was observed. Measurements on clot optical density were performed on individual serum samples, rather than pooled samples. The average clot optical density was then calculated for each study group and then presented in the graphs.



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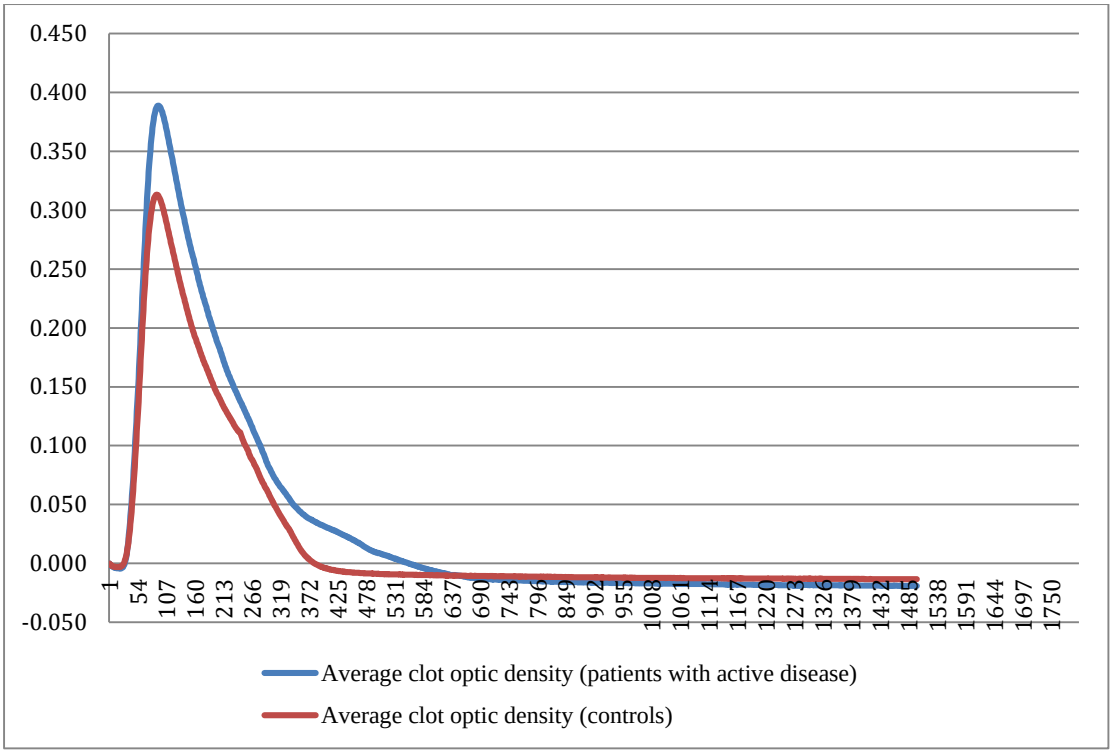
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Average clot optic density (patients with active disease)
Average clot optic density (controls)

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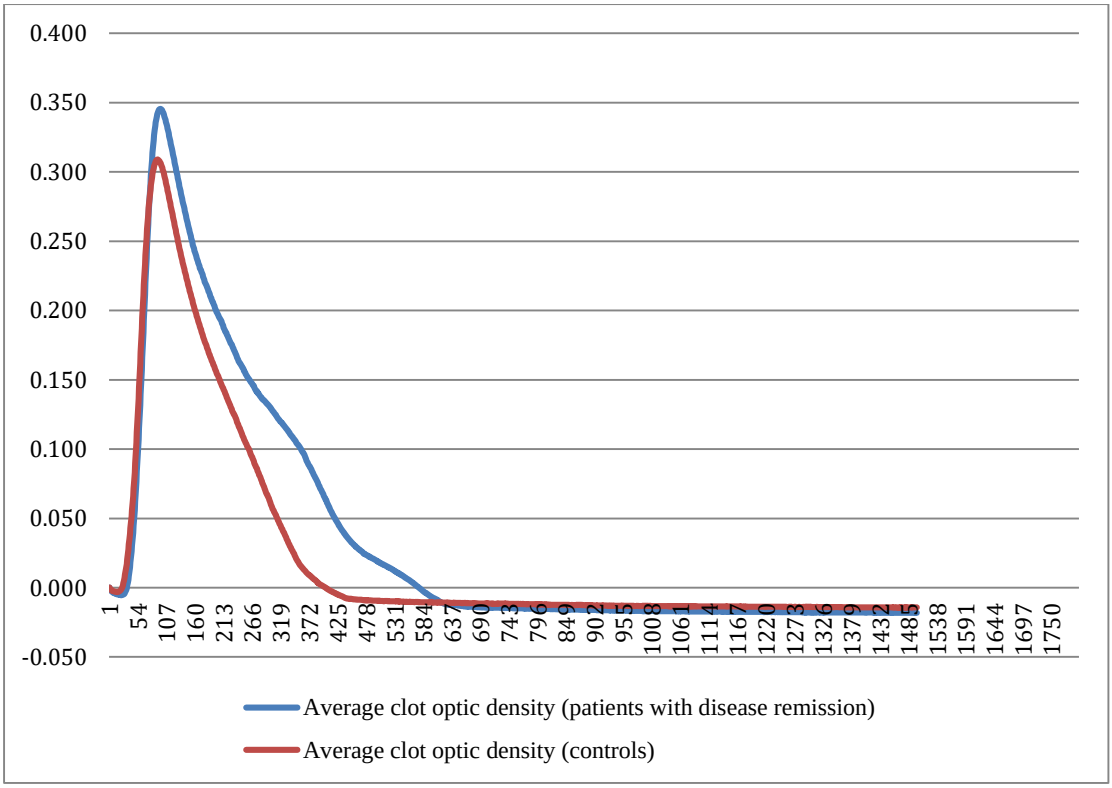
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Average clot optic density (patients with disease remission)
Average clot optic density (controls)

(iii)

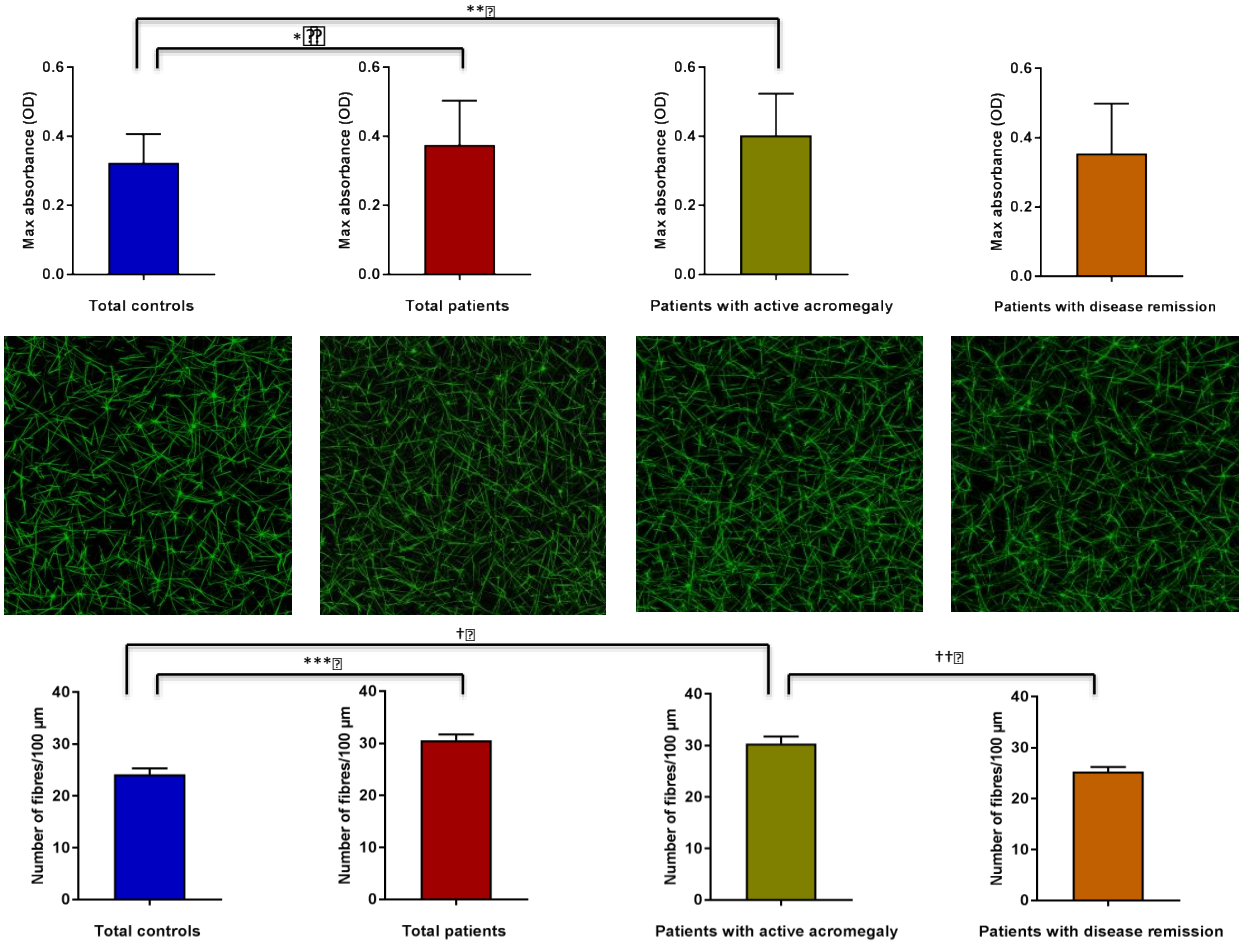
Table 5.10. Comparison in clot structure properties, fibrinogen, PAI-1 and CRP among patients with active acromegaly (n=22), patients with disease remission (n=18) and control subjects (n=40) after adjusting for BMI, using univariate analysis of covariance (ANCOVA). Results are presented as mean values with standard deviations. CRP: C reactive protein; PAI-1: plasminogen activator inhibitor-1.

	Active disease (n=22)	**Disease remission (n=18)**	**p-value**	**Active disease (n=22)**	**Controls (n=40)**	**p-value**	**Disease remission (n=18)**	**Controls (n=40)**	**p-value**
Lag time (sec)	515.6±15.1	570.2±16.8	**0.02**	519.0±17.0	528.0±12.4	0.67	572.3±19.3	529.8±12.6	0.08
Maximum optical density (arbitrary units)	0.41±0.03	0.35±0.03	0.18	0.39±0.02	0.33±0.01	**0.03**	0.345±0.03	0.33±0.02	0.6
50% Lysis time (min)	25.5±4.3	33.2±4.8	0.24	23.1±3.1	24.0±2.3	0.83	33.5±4.3	23.6±2.8	0.066
Lysis Area (arbitrary units)	849.7±130.9	837.9±144.8	0.95	748.4±97.6	607.0±72.5	0.26	834.5±99.8	590.1±65.9	0.053
Fibrinogen (mg/ml)	3.9±0.3	3.2±0.4	0.2	3.8±0.2	2.6±0.2	**<0.001**	3.25±0.2	2.6±0.15	**0.02**
PAI-1 (ng/ml)	8.7±2.4	9.1±2.6	0.9	7.2±1.8	5.6±1.3	0.49	8.5±1.6	5.6±1.1	0.15
CRP (mg/L)	11.3±5.3	15.2±5.8	0.63	8.6±4.0	11.7±3.0	0.55	13.7±5.3	11.5±3.5	0.74

5.4.6.iv. *Laser scanning confocal microscopy (LSCM)*

Patients with acromegaly were found to have a more compact fibrin clot network compared with controls (mean fibrin fibres per 100 μm 30.6±1.1 vs. 24.1±1.2 respectively, p=0.002). The subgroup of patients with active acromegaly was also noted to have a more dense fibrin network, not only compared with controls (mean fibrin fibres per 100 μm 30.4±1.3 vs. 24.1±1.2, p=0.004), but also with patients with disease remission (mean fibrin fibres per 100 μm 30.4±1.3 vs. 25.3±0.9, p=0.005). There was no difference in the density of the fibrin network of the clots of patients in remission and controls (mean fibrin fibres per 100 μm 25.3±0.9 vs. 24.1±1.2, respectively, p=0.24). These findings are consistent with the turbidimetric assay data previously presented. Images of fibrin clots from pooled samples of the entire patient cohort, as well as of the subgroup of patients with active disease, disease remission and the control group can be found in Figure 5.5.

Figure 5.5. The middle panel shows the fibrin network of clots formed ex vivo from pooled plasmas of (i) all controls (N=40); (ii) all patients with acromegaly (N=40); (iii) patients with active disease (n=22); and (iv) patients with disease remission (n=18), obtained by laser scanning confocal microscopy, in conjunction with clots maximum optical density (top panel), as calculated by turbidimetric assay and number of fibres per 100 μm (bottom panel) incorporated in the clot structure. A higher density of clot fibrin network was observed in patients with acromegaly compared with controls as also supported by the higher clot maximum optical density (*, p=0.02) and the higher number of fibres/100 μm (***, p=0.002). Patients with active acromegaly continued to demonstrate higher clot maximum absorbance (**, p=0.004) and higher number of fibres/100 μm (†, p=0.004) compared with controls, and also higher number of fibres/100 μm compared with patients with disease remission (††, p=0.005). In contrast, there was no difference in the maximum clot optical density and number of fibres/100 μm between patients with disease remission and controls.



5.5. DISCUSSION

Patients with acromegaly have increased cardiovascular mortality [179, 300, 335, 352, 477] and an increased thrombotic potential has been proposed [568, 571, 572, 595]. However, previous studies have only examined clotting and fibrinolytic factors, whereas this study has the advantage of a more global assessment of clot formation and clot lysis, providing a detailed analysis of clot structure properties, reflecting the balance between thrombotic and fibrinolytic factors, whilst at the same time taking into account the patients' metabolic profile and cardiovascular risk factors.

This study is the first to show that patients with active acromegaly have more compact clots compared with controls matched for age and gender, based on an ex-vivo clot structure analysis and after adjustment for BMI. Laser scanning confocal microscopy showed that the difference in the fibrin clot density is more prominent in the subgroup of patients with active disease, with the groups of patients with long-term disease remission and controls being essentially indistinguishable.

Similar to previous reports [568-575], this study demonstrated higher fibrinogen levels amongst patients with active acromegaly compared with healthy controls (even after adjusting for BMI), which translated into higher maximum clot absorbance (a measure of clot density) during clot formation, suggesting that patients formed more compact clots. Despite this, lysis time was not statistically different between patients and controls neither were PAI-1 levels. In contrast, there was a trend towards greater lysis area (a complex and broader measure of clot formation, clot density and lysis potential, which represents the area under the curve) in patients with acromegaly compared with control ($p=0.08$). Overall the above results suggest that patient with active acromegaly have increased thrombotic potential with increased clot density, however the fibrinolytic system does not seem to be significantly affected.

Direct comparison between patients with active acromegaly at the time of the study and those with disease remission did not reveal any significant differences in the clot formation and clot lysis properties, although the study was not powered for these outcomes. The only significant

finding was that patients with active disease had shorter lag time than patients in remission (p=0.02), suggesting that clot formation starts earlier in the former group.

However, when each patient subgroup was compared with controls, patients with active acromegaly had significantly higher fibrinogen and maximum clot optical density. These differences were not observed when patients with disease remission were compared against controls, which suggests that the increased clot density observed in the entire cohort of patients with acromegaly was mainly driven by the subgroup of patients with active disease. Following adjustment for BMI, patients with disease remission were also found to have higher fibrinogen levels compared with controls; however this did not lead in significant differences in clot MA.

In support of the above, were the results of LSCM, which showed significantly higher number of fibrin fibres per 100 μm in patients with active acromegaly compared with those in remission, suggesting the presence of increased fibrin network density in the former patient group. This implies that active acromegaly is associated with increased thrombotic potential, which may at least be partially reversed following successful treatment and biochemical control of the disease, however large prospective studies are required to confirm this hypothesis. This is consistent with the results of previous studies, which have demonstrated improvement to cardiovascular risk factors (including fibrinogen, PAI-1, blood pressure, lipid profile, insulin sensitivity index) following treatment of acromegaly [569, 577, 578].

Investigating for factors that may influence clot structure properties in patients with acromegaly, the adverse metabolic profile was associated with increased thrombotic potential in these patients. Elevated BMI, total fat mass and summative skinfold thickness were all independent risk factors for higher clot optical density, while summative skinfold thickness was also positively associated with prolonged lysis time. Elevated BMI was also associated with higher PAI-1 levels, which were subsequently strongly correlated with more prolonged lysis time, larger lysis area and overall resistance to fibrinolysis. Smoking was an independent risk factor for higher fibrinogen levels, which were also associated with higher maximum clot optical density, shorter lag time for clot formation and overall increased thrombotic potential.

Diagnoses of diabetes or impaired glucose tolerance were also independently associated with prolonged lysis time. This is consistent with the results of previous studies, which have shown that patients with diabetes have increased resistance to fibrinolysis and therefore increased thrombotic potential [596-598]. Similar associations were also observed in the control and although adverse metabolic profile was not directly related to adverse clot formation and lysis properties, it was associated with higher fibrinogen and PAI-1 levels.

It is well-recognized that acromegaly is associated with a variety of metabolic complications including impaired glucose tolerance, diabetes, hypertension and disorders of lipid metabolism and particularly hypertriglyceridaemia and reduced HDL-cholesterol [123]. This was also evident by the results of this study, in which patients with acromegaly demonstrated an adverse body composition profile with significantly higher BMI, total fat mass, waist hip ratio and triglyceride levels compared with controls, despite the fact that the control group did not consist of particularly lean individuals, with the mean BMI being in the overweight range (26.7 ± 4.1 kg/m^2), which is representative of the UK population [599]. Additionally, as expected, there was a higher prevalence of hypertension, dyslipidaemia and diabetes in the acromegaly group compared with controls, as evident by the numbers of patients who were already on medical treatment for the above conditions at the time of the study.

Considering the effect of the adverse metabolic profile and body composition on clot formation and lysis properties, it is essential that patients with acromegaly are screened and appropriately treated for these metabolic complications, but even more importantly and that acromegaly is diagnosed early in the disease course and treated successfully achieving biochemical disease control, in order to minimise duration of active disease and prevent complications from arising.

Notably, in this study, patients with disease remission continued to exhibit an adverse profile of body composition and cardiovascular risk factors, as evident by the higher WHR, fat mass, triglycerides and rates of hypertension and dyslipidaemia, and despite no difference in clot fibrin network density, this may account for the higher fibrinogen levels and the trend

towards more prolonged lysis time and larger lysis area compared with controls, following adjustment for BMI.

However, adverse body composition and diabetes are not the only factors responsible for the negative impact on clot formation properties in patients with acromegaly. Based on multiple linear regression analysis, adjusting for age, gender and metabolic parameters; longer duration of active disease was associated with higher fibrinogen levels; shorter duration of disease remission was associated with larger lysis area; and higher GH levels at the time of the study were associated with shorter lag time for clot formation, suggesting that disease activity adversely affects the thrombotic potential in patients with acromegaly, independently of the metabolic complications. Additionally, when comparing patients with active acromegaly with patients in remission, body composition and prevalence of metabolic complications were similar between the two groups, as were mean age and gender distribution. Despite the above similarities, LSCM showed more compact clots with higher concentration of fibrin fibres, which further strengthens the hypothesis that active acromegaly independently increases the thrombotic potential in these patients. This is consistent with previous all cause mortality and cardiovascular mortality data, which have shown increased SMR in patients with active acromegaly, but not in those with disease remission [179, 300, 474, 475].

Limitations to the study include the heterogeneity of the patient group, which consisted of patients with active disease as well as patients with disease remission. Additionally, patients were at different stages of their disease: 3 patients had been diagnosed close to their recruitment to the study and had received no treatment for acromegaly, whereas the remaining patients had been diagnosed with acromegaly 2-25 years prior to participating to the study and had received multi-modality treatment (pituitary surgery, radiotherapy, medical therapy) in different combinations. However, considering the rare incidence of acromegaly in the general population and that this was a single-centre study, decision was made to include every patient with acromegaly, providing that they met the inclusion/exclusion criteria as outlined in the methodology section. Although the study was powered to show differences in clot

structure properties between the entire cohort of patients compared with the entire group of controls, which was also the primary outcome of the study, it was not powered for comparisons between the subgroup of patients with active disease and the subgroup of disease remission, which may have led to a type II statistical error, in view of the relatively small number of patients in the two subgroups. However, it was possible to demonstrate the presence of more adverse clot structure properties in patients with active disease, when each subgroup was compared with their respective controls. Additionally, patients with active acromegaly had significantly higher number of fibrin fibres per 100 μm on the LSCM compared with both controls and patients with disease remission. Finally, this study assesses the cumulative effect of plasma proteins on clot formation and lysis in acromegaly and does not investigate the role of cellular components (i.e. platelets).

A significant proportion of the initially screened patients for this study (21 patients out of 92 screened, 22.8%) were excluded as they were already on treatment with antiplatelet of anticoagulant agents at the time of the study. Of those, 11 patients were on secondary prevention treatment after having sustained a significant thromboembolic event (i.e. pulmonary embolism, ischaemic stroke or myocardial infarction), while the remaining 10 patients were on primary prevention due to other cardiovascular risk factors and comorbidities (i.e. atrial fibrillation, diabetes, hypertension, dyslipidaemia). Therefore, by excluding this high-risk subgroup of acromegalic patients, it is possible that the study has underestimated the severity of the clot structure abnormalities in acromegaly and the effect of the disease on the increased thrombotic potential of these patients.

5.6. CONCLUSIONS

In conclusion, this study provides new evidence that patients with active acromegaly have abnormal clot structure properties, particularly with regards to clot formation, with higher fibrinogen levels and maximum clot density. This may represent one mechanism for the increased cardiovascular risk observed in patients with acromegaly, particularly during active disease. The effect of acromegaly on the abnormal clot structure properties is likely multifactorial, with the adverse metabolic profile observed in these patients (unfavourable body composition, diabetes), as well as disease activity being associated with the increased thrombotic potential in acromegaly. Successful treatment of acromegaly, with normalisation of GH and IGF-1, as well as appropriate management of the associated metabolic complications may reduce thrombotic potential in these patients by lowering clot MA and fibrinogen levels, however further prospective studies are required to confirm this hypothesis.

CHAPTER SIX

THESIS CONCLUSIONS AND FUTURE RESEARCH

6.1. Conclusions

Acromegaly is a rare, multi-systemic disease, with the effects of GH excess being realized in a variety of peripheral tissues and organs. Despite advancements in therapeutics interventions, including newer surgical techniques for trans-sphenoidal surgery (i.e. endoscopic approach), novel radiotherapy delivery methods (i.e. stereotactic radiotherapy, intensity-modulated radiation therapy) allowing higher radiotherapy doses to be delivered to the target lesion, while sparing surrounding healthy tissue and also introduction of newer pharmacological agents (i.e. growth hormone receptor antagonist, second generation somatostatin analogue), there remains several unresolved issues in patients with acromegaly. Even despite successful treatment and biochemical remission, these patients are often left with significant morbidity which impacts on their quality of life long-term. Of the different causes of morbidity, arthropathy has the highest prevalence amongst patients with acromegaly and is one of the main factors affecting patient's quality of life. Regarding mortality associated with acromegaly, despite recent studies showing mortality rates overall similar to the background population, there still remains an increased mortality risk due to cardiovascular disease with a collective SMR of 1.95 [179]. The unresolved issues in acromegaly described above, were explored further in this Thesis.

6.1.1. Insights into Quality of Life

In chapter 2, quality of life was assessed in a cohort of patients with acromegaly, long after their initial diagnosis and treatment with stable biochemical disease control, using the disease-specific questionnaire, AcroQoL, and a number of generic QoL questionnaires. Patients experience significantly impaired QoL in all the subscales of the questionnaires used in the study. Appearance was the most affected subscale in the AcroQoL questionnaire, while the physical subscales of energy, vitality, pain and physical limitations, were the most underscored domains in the generic QoL questionnaires. Emotional well-being and psychosocial functioning were also impaired, but to a lesser degree.

Factors associated with better QoL outcomes included longer duration of GH and IGF-1 control, female gender (although this could be due to the fact that female patients in this study had lower IGF-1 levels and longer duration of IGF-1 control compared with male patients) and older age. Gonadotrophin deficiency as a result of multi-modality treatment for acromegaly and long-term use of GH lowering medical therapy were associated with negative QoL outcomes.

Following a further QoL assessment after 5.7±0.6 years of follow-up, impaired QoL persisted and no change to the baseline QoL scores was observed, despite patients having a more favourable biochemical profile at the time of follow-up with significantly lower GH levels. Disease activity status at baseline assessment was not predictive of QoL outcomes, as no difference in QoL scores was observed between patients fulfilling the biochemical criteria for disease remission and those were still showing evidence of biochemical disease activity. However, patients who demonstrated a decline to QoL score in the AcroQoL and EQ-5D questionnaires at follow-up, had significantly higher IGF-1 values at baseline compared with patients QoL scores improved at follow-up assessment, implying that although biochemical disease status may not be associated with patients' current QoL as a numerical value, it may be predictive of QoL future improvement or decline.

6.1.2. Insights into Bone Shape as a potential marker for arthropathy in acromegaly

In Chapter 4, knee bone shape, joint space width and cartilage thickness were measured based on automated segmentation of MR images of knee bones and calculation of bone area using active appearance models and results were compared with bone shape of control group derived from the publicly available Osteoarthritis Initiative database, including individuals at risk of developing osteoarthritis and individuals without evidence of osteoarthritis based on serial MRI scans (OA-free group).

Patients with acromegaly have preserved/increased joint space width, which is due increased cartilage thickness compared with controls. Bone area was larger in patients with acromegaly

particularly at the patella and media tibia. B-score, a newly proposed biomarker for OA, was significantly higher in patients with acromegaly. Within the acromegaly group, there was a subgroup of patients (n=21, 35% of the entire cohort) with B-score ≥2, which is outside the reference range of healthy controls and has been associated with higher severity and risk of progression of joint disease in patients with OA. These patients had significantly larger bone areas, preserved joint space and increased cartilage thickness compared with patients with B-score <2. Regarding acromegaly-related features, patients with B-score ≥2 had higher GH levels at diagnosis and required higher number of therapeutic interventions during the course of the disease, suggesting that exposure to higher GH levels leads to more extensive changes to the bone shape and potentially increase the risk of arthropathy. Higher GH levels at the time of diagnosis were also found in patients who had previously undergone joint replacement surgery compared with patients without such medical history.

Differences in the bone shape between patients and controls were more pronounced for patients with B-score ≥2, while patients with B-score <2, apart from increased cartilage thickness, had almost indistinguishable bone shape compared with OAI controls. However, when patients with B-score <2 were compared against the selective OA-free control group, increased size of patella and medial tibia, alongside with increased B-score, medial JSW and cartilage thickness were found, suggestive of altered bone shape even in this seemingly healthy subgroup of acromegaly patients.

There was no difference in reported joint pain between patients with B-score ≥2 and those with B-score <2, likely due to the fact that joint space width was similar in the two groups. Preserved joint space is the main feature that seems to distinguish the acromegalic arthropathy from primary OA. In fact, acromegaly patients who were expected to have more advanced joint pathologies (i.e. patients with B-score ≥2 or patients with previous joint replacement surgery) had either preserved or increased JSW respectively, when compared with the remaining patients. No change in knee bone shape was observed at 12 months follow-up, based on repeat MRI scans.

6.1.3. Insights into Clot Structure properties as a potential factor for increased cardiovascular risk

In Chapter 5, clot formation and lysis properties were assessed in a group of 40 patients with acromegaly, which included patients with active disease, as well as patients in biochemical remission, using a validated turbidimetric assay and laser canning confocal microscopy for the visualisation of the clot fibrin network.

Patients with active acromegaly have more compact clots with higher optical density compared with controls. This was also confirmed by laser scanning confocal microscopy, which showed increased fibrin network density, with higher number of fibrin fibres per 100 μm. Patients with active acromegaly were also found to have higher fibrinogen concentration, which translated into more dense clots. In contrast, acromegaly patients with long-term disease remission had similar clot structure properties with the background population. Despite increased clot density fibrinolysis was not impaired and lysis times, as well as PAI-1 levels were similar with controls, both for patients active disease and disease remission.

Elevated BMI, total fat mass, summative skinfold thickness and diagnosis of diabetes/ impaired glucose tolerance were independent risk factors for adverse clot formation and lysis properties. In addition to those metabolic parameters, disease activity was also an independent risk factor for increased thrombotic potential in patients with acromegaly, as longer duration of active disease was associated with higher fibrinogen levels, shorter duration of disease remission was associated with larger lysis area and higher GH levels at the time of the study were associated with shorter lag time for clot formation.

Overall, patients with active acromegaly have abnormal clot structure properties, with more compact clots, thus conferring increased thrombotic risk, which may represent one mechanism for the enhanced cardiovascular risk in these patients.

6.2. Future research

6.2.1. Future research on Quality of Life

Impaired QoL in patients with acromegaly, which perists despite adequate biochemical disease control has been previously established by a number of studies. Skeletal and soft tissue changes and other complications associated with GH/IGF-1 excess, such as arthropathy and OSA may contribute to the impaired QoL of these patients, particurarly in relation to the domains of physical appearance and physical function of the QoL questionnaires. However, it has been repeatedly reported in the literature that patients with acromegaly also experience impaired emotional well-being and psychosocial functioning. Therefore, an area for further research would include evaluating the impact of psychological interventions and support in patients with acromegaly in the context of a longitudinal study.

6.2.2. Future research on Arthropathy

The arthropathy in acromegaly has received little research attention overall, despite being the commonest cause of morbidity in this particular patient group. Additional studies using modern imaging modalities (such as MRI scan) are required to further characterize the arthropathy in acromegaly. These studies should include imaging of different joint areas, such as small and large joints of the upper limbs and the different areas of the axial skeleton, given the wide distribution of joint pain in patients with acromegaly, which can essentially affect any joint area.

Furthermore, inclusion of larger number of patients with acromegaly in a study would increase the power of intragroup analyses such as those performed in the study presented in this thesis (i.e. patients with B-score ≥2 vs. patients with B-score <2; patients with previous history of joint replacement surgery vs. patient without prior joint surgery). Further sub-analyses could also be performed including comparisons between patients with active disease versus patients with disease remission or patients with joint pain versus asymptomatic patients.

Prospective studies with long duration of follow up are also required to assess the rate of progression of arthropathy in patients with acromegaly. No change in knee bone shape was found at 12 months follow-up in the study presented in Chapter 4, indicating that a longer follow-up period is needed in order to understand the natural history of joint alterations in patients with acromegaly. Such prospective studies would also allow to investigate whether joint alterations progress differently in patients who have achieved long-term disease remission, compared with patients with on-going active acromegaly. Another study endpoint could be joint replacement surgery in order to identify factors, which can be predictive of end-stage arthropathy in patients with acromegaly.

6.2.3. Future research on Clot Structure and Cardiovascular Risk

Further prospective studies are required to fully elucidate the effect of acromegaly on clot structure properties. These studies should aim to assess clot formation and lysis in patients with active acromegaly prior to any treatment and also after successful treatment and biochemical disease control. However, considering the low incidence of acromegaly, this type of prospective study would be more feasible in a multi-centre setting. Evaluation of clot structure properties at different time points following successful treatment of acromegaly would provide information not only as to whether the increased thrombotic potential of these patients reverses after treatment, but also as to the time required for this to occur after treatment. However, a caveat to this is that often patients require a long period of time for GH and IGF-1 to return to the normal range, particularly in those cases where radiotherapy has been used to treat acromegaly, which suggests that such prospective studies would need to have a long follow-up period.

A larger sample size would also allow the subgroup of patients with dichotomous GH/IGF-1 results to be assessed separately to the active disease subgroup for difference in the clot structure properties and a comparison between patients with high GH dichotomy versus high IGF-1 dichotomy would provide insight into whether the latter group is at increased vascular

risk and therefore in need for more aggressive treatment to normalise IGF-1. Additionally, prospective studies would allow evaluation of whether the unfavourable clot structure properties are linked with worse cardiovascular outcomes in patients with acromegaly.

In addition to fibrinogen levels, a number of proteins have been detected in the fibrin network that may alter properties and resistance to lysis including fibronectin, α2-antiplasmin, complement C3, histidine-rich glycoprotein and apolipoproteins [600]. These warrant further investigation to establish the exact mechanisms for altered clot structure in individuals with acromegaly.

LIST OF PUBLICATIONS AND PRESENTATIONS FROM THIS THESIS

Publications

1. Impaired quality of life in patients with treated acromegaly despite long-term biochemically stable disease: Results from a 5-year prospective study.

   Kyriakakis N, Lynch J, Gilbey SG, Webb SM, Murray RD.

   Clinical Endocrinology, 2017; 86(6): 806-815.

2. Prothrombotic fibrin network characteristics in patients with acromegaly: a novel mechanism for vascular complications.

   Kyriakakis N, Pechlivani N, Lynch J, Oxley N, Phoenix F, Seejore K, Orme SM, Ajjan R, Murray RD.

   European Journal of Endocrinology, 2020; 182(5): 511-521.

3. Management of persistent acromegaly following primary therapy: The current landscape in the UK.

   Kyriakakis N, Seejore K, Hanafy A, Murray RD.

   Endocrinology, Diabetes & Metabolism, 2020; 3(3):e00158.

Conference Presentations

1. Perceived quality of life in acromegaly: results from a tertiary UK centre. **Kyriakakis N**, Lynch J, Gilbey SG, Webb S, Murray RD.

   Poster presentation (guided poster tour) - European Congress of Endocrinology ECE 2015, Dublin, Ireland

   Endocrine Abstracts (2015) **37** GP19.03.

2. Impaired quality of life in patients with acromegaly despite long-term disease control: results from a longitudinal study.

   Kyriakakis N, Lynch J, O'Qwyer J, Gilbey SG, Murray RD.

   Poster presentation - Society for Endocrinology BES 2015, Edinburgh, UK *Endocrine Abstracts* (2015) **38** P326.

3. Impaired quality of life in patients with acromegaly despite long-term disease control: results from a longitudinal study.

   Kyriakakis N, Lynch J, Gilbey SG, Webb SM, Murray RD.

   Poster presentation - ENDO 2016, Boston, USA (competed in the Endocrine Society's Presidential Poster Competition)

4. The burden of arthropathy in acromegaly: results from an observational study. **Kyriakakis N**, Lynch J, Orme SM, Gilbey SG, Conaghan P, Murray RD. Poster presentation - Society for Endocrinology BES 2017, Harrogate, UK

   Endocrine Abstracts (2017) **50** P292.

5. Higher fibrinogen and clot density in patients with acromegaly: the role of adverse body composition to the increased thrombotic potential.

   Kyriakakis N, Lynch J, Seejore K, Phoenix F, Oxley N, Orme SM, Ajjan R, Murray RD.

   Poster presentation - ENDO 2019, New Orleans, USA (Featured poster, competed in the Endocrine Society's Presidential Poster Competition)

6. Assessing the impact of disease activity on clot formation and lysis in patients with acromegaly.

   Kyriakakis N, Lynch J, Seejore K, Phoenix F, Oxley N, Orme SM, Ajjan R, Murray RD. Poster presentation - ENDO 2019, New Orleans, USA

7. Increased clot density in patients with acromegaly: the role of the adverse metabolic profile and disease activity to the increased thrombotic potential. **Kyriakakis N**, Pechlivani N, Lynch J, Oxley N, Phoenix F, Seejore K, Orme SM, Ajjan R, Murray RD.

   Poster presentation - Society for Endocrinology BES 2019, Brighton, UK

   Endocrine Abstracts (2019) **65** P271.

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Appendix I: Samples of the questionnaires for the assessment of quality of life in patients with acromegaly

ACROMEGALY- QUALITY OF LIFE QUESTIONNAIRE

(ACROQoL)

Today's date Day Month Year

INSTRUCTIONS FOR ANSWERING THE QUESTIONNAIRE

In the following pages there are sentences that describe some of the problems that acromegaly causes to people who, like you, suffer from this illness.

Each sentence is followed by some response options. Some of these refer to the **frequency**, while others refer to **how much you agree or disagree** with them.

Please, read each sentence carefully. Then tick the response option which best describes what you think is happening to you.

Remember that there are NO correct or incorrect answers. We are only interested in what is currently happening to you because of your acromegaly.

It is very important to answer all the questions.

Thank you very much for your collaboration

Because of my Acromegaly….

1. My legs feel weak

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

2. I feel ugly

- Completely agree ☐
- Moderately agree ☐
- Neither agree nor disagree ☐
- Moderately disagree ☐
- Completely disagree ☐

3. I get depressed

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

4. I look awful in photographs

- Completely agree ☐
- Moderately agree ☐
- Neither agree nor disagree ☐
- Moderately disagree ☐
- Completely disagree ☐

5. I avoid going out very much with friends because of my appearance

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

6. I try to avoid socialising

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

Because of my Acromegaly...

7. I look different in the mirror

- Completely agree ☐
- Moderately agree ☐
- Neither agree nor disagree ☐
- Moderately disagree ☐
- Completely disagree ☐

8. I feel rejected by people because of my illness

- Completely agree ☐
- Moderately agree ☐
- Neither agree nor disagree ☐
- Moderately disagree ☐
- Completely disagree ☐

9. I have problems carrying out my usual activities (e.g. working, studying, doing household tasks, family or leisure activities)

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

10. People stare at me because of my appearance

- Completely agree ☐
- Moderately agree ☐
- Neither agree nor disagree ☐
- Moderately disagree ☐
- Completely disagree ☐

11. Some parts of my body (nose, feet hands,...) are too big

- Completely agree ☐
- Moderately agree ☐
- Neither agree nor disagree ☐
- Moderately disagree ☐
- Completely disagree ☐

12. I have problems doing things with my hands, for example, sewing or handling tools

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

Because of my Acromegaly….

13. The illness affects my performance at work or in my usual tasks

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

14. My joints ache

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

15. I feel tired

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

16. I snore at night

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

17. It is hard for me to articulate words due to the size of my tongue

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

18. I have problems with sexual relationships

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

Because of my Acromegaly….

19. I feel like a sick person

- Completely agree ☐
- Moderately agree ☐
- Neither agree nor disagree ☐
- Moderately disagree ☐
- Completely disagree ☐

20. The physical changes produced by my illness govern my life

- Completely agree ☐
- Moderately agree ☐
- Neither agree nor disagree ☐
- Moderately disagree ☐
- Completely disagree ☐

21. I have little sexual appetite

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

22. I feel weak

- Always ☐
- Most of the time ☐
- Sometimes ☐
- Rarely ☐
- Never ☐

Finally, please check that you have answered all the questions.

Once again thank you very much for your collaboration.

The Psychological General Well-Being Schedule

This questionnaire asks you about how you have been feeling and how things have been going for you, DURING THE PAST MONTH. For each question please tick [✓] the answer which best applies to you. Please answer ALL the questions.

1. How have you been feeling in general? (DURING THE PAST MONTH)

[ ] in excellent spirits
[ ] in very good spirits
[ ] in good spirits mostly
[ ] I have been up and down in spirits a lot
[ ] in low spirits mostly
[ ] in very low spirits

2. How often were you bothered by any illness, bodily disorder, aches or pains? (DURING THE PAST MONTH)

[ ] every day
[ ] almost every day
[ ] about half of the time
[ ] now and then, but less than half the time
[ ] rarely
[ ] none of the time

3. Did you feel depressed? (DURING THE PAST MONTH)

[ ] yes - to the point that I felt like taking my life
[ ] yes - to the point that I did not care about anything
[ ] yes - very depressed almost every day
[ ] yes - quite depressed now and then
[ ] yes - a little depressed now and then
[ ] no - never felt depressed at all

4. Have you been in firm control of your behaviour, thoughts, emotions, or feelings? (DURING THE PAST MONTH)

[ ] yes, definitely so

[ ] yes, for the most part

[ ] generally so

[ ] not too well

[ ] no, and I am somewhat disturbed

[ ] no, and I am very disturbed

5. Have you been bothered by nervousness or your "nerves"? (DURING THE PAST MONTH)

[ ] extremely so - to the point where I could not work / take care of things

[ ] very much so

[ ] quite a bit

[ ] some - enough to bother me

[ ] a little

[ ] not at all

6. How much energy, pep, or vitality did you have or feel? (DURING THE PAST MONTH)

[ ] very full of energy - lots of pep

[ ] fairly energetic most of the time

[ ] my energy level varied quite a bit

[ ] generally low in energy or pep

[ ] very low in energy or pep most of the time

[ ] no energy or pep at all - I felt drained, sapped

7. I felt downhearted and blue (DURING THE PAST MONTH)

[ ] none of the time

[ ] a little of the time

[ ] some of the time

[ ] a good bit of the time

[ ] most of the time

[ ] all of the time

8. Were you generally tense or did you feel any tension? (DURING THE PAST MONTH)

[ ] yes - extremely tense, most or all of the time

[ ] yes - very tense most of the time

[ ] not generally tense, but did feel fairly tense several times

[ ] I felt a little tense a few times

[ ] my general tension level was quite low

[ ] I never felt tense or any tension at all

9. How happy, satisfied, or pleased have you been with your personal life? (DURING THE PAST MONTH)

[ ] extremely happy - could not have been more satisfied or pleased

[ ] very happy most of the time

[ ] generally satisfied - pleased

[ ] sometimes fairly happy, sometimes fairly unhappy

[ ] generally dissatisfied or unhappy

[ ] very dissatisfied or unhappy most or all of the time

10. Did you feel healthy enough to carry out things you like to do or had to do? (DURING THE PAST MONTH)

[ ] yes - definitely so

[ ] for the most part

[ ] health problems limited me in some important ways

[ ] I was only healthy enough to take care of myself

[ ] I needed some help in taking care of myself

[ ] I needed someone to help me with most or all of the things I had to do

11. Have you felt sad, discouraged, hopeless, or had so many problems that you wondered if anything was worthwhile? (DURING THE PAST MONTH)

[ ] extremely so - to the point that I have just about given up

[ ] very much so

[ ] quite a bit

[ ] some - enough to bother me

[ ] a little bit

[ ] not at all

12. I woke up feeling fresh and rested (DURING THE PAST MONTH)

[ ] none of the time
[ ] a little of the time
[ ] some of the time
[ ] a good bit of the time
[ ] most of the time
[ ] all of the time

13. Have you been concerned, worried, or had any fears about your health? (DURING THE PAST MONTH)

[ ] extremely so
[ ] very much so
[ ] quite a bit
[ ] some, but not a lot
[ ] practically never
[ ] not at all

14. Have you had any reason to wonder if you were losing your mind, or losing control over the way you act, talk, think, feel or of your memory? (DURING THE PAST MONTH)

[ ] not at all
[ ] only a little
[ ] some - but not enough to be concerned or worried about
[ ] some and I have been a little concerned
[ ] some and I am quite concerned
[ ] yes, very much so and I am very concerned

15. My daily life was full of things that were interesting to me (DURING THE PAST MONTH)

[ ] none of the time
[ ] a little of the time
[ ] some of the time

[ ] a good bit of the time

[ ] most of the time

[ ] all of the time

16. Did you feel active, vigorous, or dull, sluggish? (DURING THE PAST MONTH)

[ ] very active, vigorous every day

[ ] mostly active, vigorous - never really dull, sluggish

[ ] fairly active, vigorous - seldom dull, sluggish

[ ] fairly dull, sluggish - seldom active, vigorous

[ ] mostly dull, sluggish - never really active, vigorous

[ ] very dull, sluggish every day

17. Have you been anxious, worried, or upset? (DURING THE PAST MONTH)

[ ] extremely so - to the point of being sick or almost sick

[ ] very much so

[ ] quite a bit

[ ] some - enough to bother me

[ ] a little bit

[ ] not at all

18. I was emotionally stable and sure of myself (DURING THE PAST MONTH)

[ ] none of the time

[ ] a little of the time

[ ] some of the time

[ ] a good bit of the time

[ ] most of the time

[ ] all of the time

19. Did you feel relaxed, at ease or high strung, tight, or keyed-up? (DURING THE PAST MONTH)

[ ] felt relaxed and at ease the whole month

[ ] felt relaxed and at ease most of the time

[ ] generally felt relaxed but at times felt fairly high strung

[ ] generally felt high strung but at times felt fairly relaxed

[ ] felt high strung, tight, or keyed-up most of the time

[ ] felt high strung, tight, or keyed-up the whole month

20. I felt cheerful, light-hearted (DURING THE PAST MONTH)

[ ] none of the time

[ ] a little of the time

[ ] some of the time

[ ] a good bit of the time

[ ] most of the time

[ ] all of the time

21. I felt tired, worn out, used up, or exhausted (DURING THE PAST MONTH)

[ ] none of the time

[ ] a little of the time

[ ] some of the time

[ ] a good bit of the time

[ ] most of the time

[ ] all of the time

22. Have you been under or felt you were under any strain, stress, or pressure? (DURING THE PAST MONTH)

[ ] yes - almost more than I could bear or stand

[ ] yes - quite a bit of pressure

[ ] yes, some - more than usual

[ ] yes, some - but about usual

[ ] yes - a little

[ ] not at all

36-item Short Form (SF36)

Your Health and Well Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. *Thank you for completing this survey!*

For each of the following questions, please mark an X in the one box that best describes your answer.

1. In general, you would say your health is

☐ Excellent

☐ Very Good

☐ Good

☐ Fair

☐ Poor

2. Compared to one year ago, how would you rate your general health now?

☐ Much better now than one year ago

☐ Somewhat better now than one year ago

☐ About the same as one year ago

☐ Somewhat worse now than one year ago

☐ Much worse now than one year ago

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, by how much?

	Yes limited a lot	Yes limited a little	No, not limited at all
Vigorous activity, e.g. running, lifting heavy objects, participating in strenuous sports			
Moderate activity, e.g. moving a table, pushing a vacuum cleaner, bowling or playing golf			
Lifting or carrying groceries			
Climbing several flights of stairs			
Climbing one flight of stairs			
Bending, kneeling or stooping			
Walking more than a mile			
Walking several blocks			
Walking one block			
Bathing or dressing yourself			

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	Yes	No
Cut down the amount of time you spent on work or other activities		
Accomplished less than you would like		
Were limited in the kind of work or other activities		
Had difficulty performing the work or other activities (for example, it took extra effort)		

5. During the past 4 weeks, have you had any of the following problems with your work or other regular activities as a result of any emotional problems (such as feeling depressed or anxious)?

	Yes	No
Cut down the amount of time you spent on work or other activities		
Accomplished less than you would like		
Did work or other activities less carefully than usual		

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

☐ Not at all

☐ Slightly

☐ Moderately

☐ Quite a lot

☐ Extremely

7. How much bodily pain have you had during the last 4 weeks?

☐ None

☐ Very mild

☐ Mild

☐ Moderate

☐ Severe

☐ Very severe

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

☐ Not at all

☐ A little bit

☐ Moderately

☐ Quite a bit

☐ Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks….

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
Did you feel full of pep?						
Have you been a very nervous person?						
Have you felt so down in the dumps nothing could cheer you up?						
Have you felt calm and peaceful?						
Did you have a lot of energy?						
Have you felt down heartened and blue?						
Did you feel worn out?						
Have you been a happy person?						
Did you feel tired?						

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc)?

☐ All of the time

☐ Most of the time

☐ Some of the time

☐ A little of the time

☐ None of the time

11. How TRUE or FALSE is each of the following statements for you?

	Definitely true	Moderately true	Don't know	Mostly false	Definitely false
I seem to get sick a little easier than other people					
I am as healthy as anybody I know					
I expect my health to get worse					
My health is excellent					

EQ - 5D

Health Questionnaire

English version for the UK
(validated for use in Eire)

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about	❑
I have some problems in walking about	❑
I am confined to bed	❑

Self-Care

I have no problems with self-care	❑
I have some problems washing or dressing myself	❑
I am unable to wash or dress myself	❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities	❑
I have some problems with performing my usual activities	❑
I am unable to perform my usual activities	❑

Pain/Discomfort

I have no pain or discomfort	❑
I have moderate pain or discomfort	❑
I have extreme pain or discomfort	❑

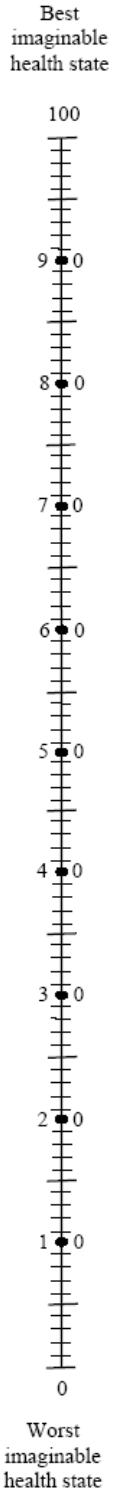
Anxiety/Depression

I am not anxious or depressed	❑
I am moderately anxious or depressed	❑
I am extremely anxious or depressed	❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today



Appendix II: Clinician case report form for data collection

The Leeds Teaching Hospitals NHS
NHS Trust

The Acromegalic Arthropathy Study

Clinician Case Report Form
Baseline Visit

Changes in the bone shape in patients with acromegaly: an MRI study

Chief Investigator
Dr Robert Murray

Participant ID:

Date:

Participant ID: Date completed:

Section A – STUDY ELIGIBILITY SCREENING

INCLUSION CRITERIA

Does the participant meet the following inclusion criteria?		
Participant must meet all of the following criteria to be considered for enrolment into the study	**YES**	**NO**
Patient has or had diagnosis of acromegaly		
Patient is ≥ 18 years of age		
Patient is capable of understanding and signing an informed consent form		

EXCLUSION CRITERIA

Does the patient meet any of the exclusion criteria?		
Patients with any of the following will not be included in the study.	**YES**	**NO**
Age less than 18 years old		
Unwilling or unable to provide informed consent		
Any diagnosis of primary inflammatory arthritis, including rheumatoid arthritis, gout, polymyalgia or connective tissue disease that can potentially affect the knee joints		
Patients with any of the following contraindications to MRI scan: ○ Pacemaker ○ Surgical clips within the head ○ Certain inner ear implants ○ Neuro-electrical stimulators ○ Metal fragments within the eye or head ○ Pregnant or breastfeeding women ○ Relative contraindication: claustrophobia, patient unable to tolerate		

Is the patient eligible for recruitment? **YES** ☐ **NO** ☐

_____ _____ _____

Investigator's Name (please PRINT) **Signature** **Date**

Participant ID: Date completed:

Section B – DEMOGRAPHIC DATA

Date of Birth | | | | **Gender** Female ☐ Male ☐

Ethnicity

Caucasian ☐ South East Asian ☐

Asian ☐ Afro-Caribbean ☐

SOCIAL HISTORY

Smoking status: Never ☐ Current ☐ Previous ☐

Number of pack years | | | |

Alcohol intake (units per week): | | | |

EMPLOYMENT HISTORY

Current status: Employed ☐ Self-employed ☐ Unemployed ☐ Retired ☐

If employed: Full time ☐ Part time ☐

Job or Activity Type (current or previous)

☐ Heavy manual ☐ Repetitive use of hands

☐ Prolonged keyboarding ☐ Prolonged standing

☐ None

Section C – ACROMEGALY HISTORY AND RELEVANT INFORMATION

1. Date acromegaly diagnosed? _____

2. Date of onset of acromegalic symptoms*? _____
   *Ask the patient about when they noticed changes in the facial appearance, increase in the size of hands and feet. Ask for old photographs in case of uncertainty.

3. Date acromegaly remission was achieved**? _____
   ** This is equal to biochemical control of the disease, defined as normalization of serum IGF-I levels and GH < 2miu/l

4. Duration of active disease***: ☐☐ years ☐☐ months
   *** Defined as the period from the start of symptoms and signs of acromegaly until normalization of serum IGF-I levels and GH < 2miu/l.

5. Duration of disease remission+: ☐☐ years ☐☐ months
   + Defined as the period from the date of normalization of serum IGF-I levels and GH < 2miu/l until the present study date.

6. GH level at the time of diagnosis: _____

7. IGF-I level at the time of diagnosis: _____

8. Type of treatment for acromegaly (tick all the applicable options):
   - **Surgical** ☐
     - **Transcranial surgery** ☐
       Times the patient had surgery ☐
       Date(s) of operation(s) _____
     - **Transphenoidal surgery** ☐
       Times the patient had surgery ☐
       Date(s) of operation(s) _____
   - **Cranial radiotherapy** ☐
     - **Conventional radiotherapy** ☐
       Greys: ☐☐
       Date: _____
     - **Stereotactic radiotherapy** ☐
       Greys: ☐☐
       Date: _____

- **Medical Treatment** ☐

  Complete as many as applicable

	Name of drug	Date started	Dated stopped	Reason stopped	Tick if ongoing
Drug 1					
Drug 2					
Drug 3					
Drug 4					
Drug 5					

9. Has the patient developed hypopituitarism? YES ☐ NO ☐

If YES, indicate the axi(e)s affected:

- Hypothalamus-Piruitary-Adrenal: YES ☐ NO ☐

  Date diagnosed _____ Treatment & Dose: _____

- Hypothalamus-Pituitary-Gonads: YES ☐ NO ☐

  Date diagnosed: _____ Treatment & Dose: _____

- Hypothalamus-Pituitary-Thyroid: YES ☐ NO ☐

  Date diagnosed: _____ Treatment & Dose: _____

- Posterior Pituitary (ADH deficiency): YES ☐ NO ☐

  Date diagnosed: _____ Treatment & Dose: _____

Section D - MEDICAL HISTORY

	History of disease		Stable or inactive at present	
	YES	NO	YES	NO
Hypertension	☐	☐	☐	☐
Hypercholesterolaemia	☐	☐	☐	☐
Ischaemic heart disease	☐	☐	☐	☐
Other cardiovascular disease	☐	☐	☐	☐
If YES, please specify:	☐	☐	☐	☐
Cerebrovascular disease	☐	☐	☐	☐
Peripheral vascular disease	☐	☐	☐	☐
Asthma	☐	☐	☐	☐
If YES and ongoing, is disease moderate/severe requiring treatment with corticosteroids	☐	☐	☐	☐
Emphysema/ chronic bronchitis	☐	☐	☐	☐
Other pulmonary disease	☐	☐	☐	☐
If YES, please specify:	☐	☐		
Diabetes	☐	☐	☐	☐
If YES, insulin dependent	☐	☐		
tablet controlled	☐	☐		
diet controlled	☐	☐		
Peptic ulcer disease	☐	☐	☐	☐
Gastro-oesophageal reflux disease	☐	☐	☐	☐
Inflammatory bowel disease	☐	☐	☐	☐
If YES and ongoing, is disease moderate/severe requiring treatment with corticosteroids	☐	☐		

	History of disease		Stable or inactive at present	
	YES	NO	YES	NO
Other GI disease	☐	☐	☐	☐
If YES, please specify:	☐	☐		
Renal disease	☐	☐	☐	☐
Chronic liver disease	☐	☐	☐	☐
Epilepsy	☐	☐	☐	☐
Nervous system disease	☐	☐	☐	☐
If YES, please specify:	☐	☐		
Depression	☐	☐	☐	☐
Endocrine disease e.g. thyroid	☐	☐	☐	☐
If YES, please specify:	☐	☐		
Inflammatory arthritis	☐	☐	☐	☐
If YES, please specify:	☐	☐		
Allergies	☐	☐	☐	☐
If YES, please specify				
Cancer	☐	☐	☐	☐
If YES, list sites and date of last treatment	Site:		Date:	
	Site:		Date:	
	Site:		Date:	

	History of disease		Stable or inactive at present	
	YES	**NO**	**YES**	**NO**
Surgery (**NOT joint related**)	☐	☐		
If YES, please specify:	1.			
	2.			
	3.			
	4.			
	5.			
Other Medical Conditions	☐	☐		
If YES, please specify:	1.		☐	☐
	2.		☐	☐
	3.		☐	☐
	4.		☐	☐
	5.		☐	☐

Section E - ARTHROPATHY HISTORY

1. Duration of knee joint pain as reported by participant: ☐☐ year(s) ☐☐ months

2. Family history of arthritis

a) Osteoarthrtis YES ☐ No ☐ If YES, 1st degree relative ☐ >2nd degree relative ☐

b) Rheumatoid Arthritis YES ☐ No ☐ If YES, 1st degree relative ☐ >2nd degree relative ☐

c) Other inflammatory arthritis YES ☐ No ☐ If YES, 1st degree relative ☐ >2nd degree relative ☐

JOINT SURGERY

Joint replacement or fusion **Yes** ☐ **No** ☐

If YES, please specify	**Site**	**Reason for surgery**	
		Osteoarthritis	**Trauma or fracture**
		☐	☐
		☐	☐

Section F - MEDICATION HISTORY

SYSTEMIC THERAPY

Medication	Dose	Frequency	Indication

If on patient on PRN analgesics please indicate frequency:

Has the patient used the medication more than half the days the previous months? YES ☐

NO ☐

Participant ID: Date completed:

LOCAL THERAPY

UPPER LIMB	Physiotherapy	Self Reported Exercise	Heat / Ice Rx	Topical NSAIDs	Topical Capsaicin	TENS machine	Acupuncture	IA steroid	Occupational therapy
Knee Osteoarthritis	☐	☐	☐	☐	☐	☐	☐	☐	☐

Section G - CLINICAL EXAMINATION

ANTHROPOMETRIC MEASUREMENTS

Weight: ☐☐☐ Kg BP: ☐☐☐/☐☐☐ mmHg

Height: ☐☐☐ m

JOINT EXAMINATION

RIGHT knee examination		
Sign/Symptom	**Clinical Features**	
Pain	With knee flexion	YES ☐ NO ☐
	With knee extension	YES ☐ NO ☐
	With knee rotation	YES ☐ NO ☐
Crepitations	With knee flexion	YES ☐ NO ☐
	With knee extension	YES ☐ NO ☐
	With knee rotation	YES ☐ NO ☐
Compartmental Tenderness (severity 0-3)	Medial tibiofemoral (MTF)	0 ☐ 1 ☐ 2 ☐ 3 ☐
	Lateral tibiofemoral (LTF)	0 ☐ 1 ☐ 2 ☐ 3 ☐
	Patellofemoral (PF)	0 ☐ 1 ☐ 2 ☐ 3 ☐
Presence of effusion (grading system 0-3)	0 (no effusion) ☐	
	1 (positive bulge sign) ☐	
	2 (positive fluctuance) ☐	
	3 (tense effusion) ☐	

Participant ID: Date completed:

LEFT knee examination					
Sign/Symptom	**Clinical Features**				
Pain	With knee flexion	YES ☐		NO ☐	
	With knee extension	YES ☐		NO ☐	
	With knee rotation	YES ☐		NO ☐	
Crepitations	With knee flexion	YES ☐		NO ☐	
	With knee extension	YES ☐		NO ☐	
	With knee rotation	YES ☐		NO ☐	
Compartmental Tenderness (severity 0-3)	Medial tibiofemoral (MTF)	0 ☐	1 ☐	2 ☐	3 ☐
	Lateral tibiofemoral (LTF)	0 ☐	1 ☐	2 ☐	3 ☐
	Patellofemoral (PF)	0 ☐	1 ☐	2 ☐	3 ☐
Presence of effusion (grading system 0-3)	0 (no effusion) ☐				
	1 (positive bulge sign) ☐				
	2 (positive fluctuance) ☐				
	3 (tense effusion) ☐				

RANGE OF MOVEMENT

Measurements to be obtained by hand goniometry of the knee joints.

	Knee Flexion (range of movement 0-150°)	**Knee Extension** (full extension at 0°, positive values in knee hyperextension, negative values if knee extension reduced)
Right knee		
Left knee		

Section H - QoL QUESTIONNAIRE

Have the participant's questionnaires been given to the patient (along with an envelope and a stamp)?

YES ☐ NO ☐

If NO to any of the above, please explain the reason:

Participant ID: Date completed:

Section I - MRI KNEES

Has an MRI of both knees been organised?YES ☐ NO ☐

Provisional date of the MRI (if known):

If NO, please explain the reason:

Section J - BLOOD TESTS

Have the following blood tests been requested (tick the appropriate answer)?

Investigation	YES	NO
GH		
IGF-I		
FSH		
LH		
Testoterone (men only)		
Oestradiol (women only)		
TSH		
Free T4		
Cortisol		
PTH		
Vitamin D		
Calcium		
Phosphate		
Magnesium		
Alkaline Phosphatase (bone isoenzyme)		

If NO, please explain the reason: