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Hypersensitivity pneumonitis and metalworking fluids contaminated by mycobacteria

To the Editors:

We read with interest the article published by TILLIE-LEBLOND *et al.* [1] relating to hypersensitivity pneumonitis (HP) in French automobile workers exposed to metalworking fluids (MWFs). Our group was involved in the UK outbreak investigation referenced in their article [2, 3], and have a clinical and research interest in this area.

Whilst TILLIE-LEBLOND *et al.* [1] are correct in stating that the majority of MWF-HP outbreaks have occurred in the USA, the UK Powertrain and French outbreaks are not the only ones to have occurred in Europe. We have provided scientific support to three other similar outbreaks in the UK, all with confirmed cases of MWF-HP [4, 5]. In addition, we have diagnosed single cases of MWF-HP or asthma in workers from at least five other workplaces close to our occupational lung disease clinic. We are also aware of a published respiratory outbreak from Croatia [6], and have recently had separate personal communications with groups in Germany and Sweden relating to investigations of possible MWF outbreaks. Supportive evidence for a more widespread European problem come from cross-sectional studies that have demonstrated an excess of respiratory symptoms and asthma in machine shop workers in southern Finland [7], and an excess of wheeze, chronic bronchitis, chronic rhinitis and eye irritation in Swedish metalworkers [8].

It is clear, therefore, that this remains an important area of research relevant to European MWF-exposed workers, as the exact aetiology of MWF-HP has remained elusive [9] since BERNSTEIN *et al.* [10] described the first cases in 1995. Although *Mycobacterium immunogenum* have been implicated as the cause in the French outbreak and a number of outbreaks in the USA, there is strong evidence against this being the cause of the UK outbreaks [3, 5]. In the UK Powertrain investigation, it was not possible to culture any opportunuistic mycobacteria or find any evidence of mycobacterial DNA by PCR in 125 MWF samples. Analysis of MWF samples from two other workplaces associated with UK MWF-HP outbreaks also found no detectable mycobacterial DNA. In addition to this, no demonstrable precipitin responses to extracts of *M. immunogenum*, *Mycobacterium chelonae* or *Mycobacterium fortuitum* were seen in 129 Powertrain UK workers, a group that included 17 cases of MWF-HP, 70 cases of occupational asthma, and 42 asymptomatic exposed controls. Further evidence against a mycobacterial cause came from specific challenges performed in two workers, where positive responses were seen after controlled exposure to used MWFs that did not contain mycobacteria [3].

Although referenced by TILLIE-LEBLOND *et al.* [1], the detailed immunological investigation performed on workers from a MWF-HP outbreak in the USA, where mycobacterial contamination was identified [11], is not discussed in any detail. In this key study [11], *in vitro* secretion of interleukin-8, tumour necrosis factor-α and interferon-γ were measured in whole blood and from peripheral blood mononuclear cells in response to incubation with *M. immunogenum* antigen. These measures of immunity to *M. immunogenum* were able to distinguish between MWF-exposed and -unexposed workers, but not between workers with and without MWF-HP. This study serves to highlight the difficulties of interpreting immunological findings in HP, as many workers with demonstrable immune responses are asymptomatic, and never go on to develop disease [12].

TILLIE-LEBLOND *et al.* [1] state in their article that the presence of five arcs to *M. immunogenum* by electrosyneresis differentiates MWF-HP from healthy exposed workers. Whilst this may have been true in most cases, it was not true for all, and may therefore be of limited clinical value in isolation. It should be noted that the two groups in this study had also been differentiated in other ways, based on the presence of symptoms, lung crackles and normal gas transfer prior to any immunological comparison. Whilst we agree with the authors that this test threshold needs further validation, there seems little point in aiming to differentiate those with MWF-HP from healthy workers without symptoms, as this can be done more easily by questionnaire. Whether the five-arc threshold has any diagnostic value is dependant on whether it can assist in differentiating workers experiencing work-related symptoms due to allergic occupational lung disease (HP and asthma) from those with similar symptoms due to chronic bronchitis, exacerbations of existing respiratory conditions, nonspecific irritant responses or humidifier fever. Alternatively, the utility of the test in preventing disease, as part of a prospective study of health surveillance linked to exposure modification in MWF apprentices, would also be of great interest. It is these areas that we believe merit further study by the authors to continue to improve the knowledge base in the complex area of MWF-HP outbreaks.

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From the authors:

It is widely recognised that cases of hypersensitivity pneumonitis (HP) occurring in metalworking fluids (MWFs) workers in the literature tend to be more often described in Europe [1, 2]. In certain cases, difficulty in gaining access to the samples of some companies (related to the reluctance of employers) and the lack of knowledge of MWF-HP can also lead to underdiagnosis. We agree with C.M. Barber and colleagues that this is an important subject that deserves evaluation at the European level and a large-scale prevention policy covering workshop design and the composition of MWF. In France, we are continuing with microbiological evaluation of MWF-HP and have investigated two further large car engine factories and 14 micromechanics companies (STEFI study (*Santé au Travail et Exposition aux Fluides de coupe Industriels*)) since our article was published [3].

C.M. Barber and colleagues evoke the elusive nature of the exact aetiology of MWF-HP whilst noting that, in the UK investigation at the Powertrain UK metalworking site (Birmingham, UK), it was not possible to culture any opportunistic mycobacteria or find any evidence of mycobacterial DNA by PCR of 125 MWF samples [2, 4]. In our series, similarly to a number of US outbreaks, *Mycobacterium immunogenum* has been implicated as the cause of the MWF-HP. Evidence of the causative role of *M. immunogenum* was further strengthened by two animal-model studies published in 2006. These demonstrated that the disease was induced in the mouse by repeated nasal instillation of lysates of *M. immunogenum* or MWF contaminated by *M. immunogenum* [5, 6]. Finally, there are few studies like ours [3] that simultaneously identify both a possible antigen in the MWF and the precipitins. Indeed, it has been emphasised that the demonstration of the presence of precipitin is a major factor in facilitating the diagnosis of HP, even if such precipitins are sometimes also present in exposed asymptomatic people [7].

Regarding the differences in culture efficacy and microbiological identification, for the two new car factories investigated in France, we have again cultured *M. immunogenum* on Müller–Hinton and on Middlebrook 7H10 agars. It should be noted that, in case of intense growth, samples were either serially diluted or decontaminated. Rods morphologically consistent with the genus *Mycobacterium* were identified by amplification of partial *hsp 65* gene and sequenced using the previously described primers Tb11 and Tb12. Our current work (unpublished data; G. Reboux, personal communication) compares three car factories and 14 micromechanics companies. ~30% of samples in the first (n=83 aqueous samples) and second car factory (n=44 aqueous samples) were positive for *M. immunogenum*. In the third factory (n=38 aqueous samples), which had undergone intensive treatment with biocides, only two samples were positive when cultured and none were positive from the micromechanics factories [8]. This shows that the isolation of *M. immunogenum* also depends on the treatment of MWF carried out at the factory.

Our study [3] discussed the threshold value of five arcs to differentiate MWF-HP patients from healthy exposed subjects. For *M. immunogenum* the threshold of discrimination between ill and asymptomatic exposed subjects was fairly high (five arcs). Nevertheless, we agree that a threshold can always be questioned, even if it shows good sensitivity and specificity. Thus, one of the healthy exposed subjects in our series had a value of 12 arcs, which was one of the highest levels in our series. The existence of exposed subjects with precipitins has been known for some time. The level of evaluation that differentiates ill from healthy subjects depends on the immunological techniques used and the use of the antigen to which the subjects have actually been exposed. Our laboratories routinely use a panel of between three and 12 antigens for each profession and set the thresholds per antigen with respect to groups of asymptomatic and ill subjects [9]. Electrosyneresis