VEGF in idiopathic ILD

Simler et al raise an interesting possibility of the prognostic value of plasma VEGF in interstitial lung disease.1 Meyer et al2 in a previous study did not find any difference in serum VEGF levels in patients with diffuse parenchymal lung disease and those with normal smokers and patients with pulmonary fibrosis. Am J Respir Crit Care Med 2002;166:382–5.


Authors’ reply

Dr Medford laudably highlights the interesting findings of other authors regarding levels of vascular endothelial growth factor (VEGF) in idiopathic interstitial pneumonia (IIP). Indeed, our data reflect the findings of Meyer et al who studied 11 patients with IIP.3 We extend their observations in a larger cohort of patients (n = 49) and specifically relate plasma VEGF levels to disease progression and extent of fibrosis on HRCT scanning.4 Indeed, HRCT scanning is perhaps the most reliable surrogate for the extent of disease.5 Like Meyer et al, we observed reduced BAL fluid VEGF levels in patients with IIP (91 pg/ml) compared with controls (204 pg/ml). The reduction in the BAL fluid level of VEGF may reflect the absence of angiogenesis in that specific part of the lung, with the plasma VEGF level identifying a secondary phenomenon of compensatory angiogenesis in alternative areas of the lung. Alternatively, VEGF levels appear to be higher in epithelial surface fluid than in the serum, suggesting vectorial intranarial secretion and the existence of a concentration gradient from air spaces to intravascular spaces.6

Thickett et al7 are correct in their quotation of the normal range for VEGF in plasma (36–76 pg/ml) as measured by the R&D Systems Quantikine kit. They point out that this range is quoted by the kit manufacturers and is consistent with their own data and, indeed, with their own quoted range in their study.8 We did not use the Quantikine kit in this study but stated clearly that: “The ELISA capture and detection antibodies for assaying IL-8 and VEGF were selected paired reagents optimised for ELISA from R&D Systems”. R&D currently sell these paired reagents under the name “Duoset”. Different ELISA formats for VEGF quantitation using recombinant VEGF165 as standard are available. Capture reagent: (1) rabbit polyclonal antiVEGF (in house); (2) soluble flt-1 (Sflt-1); (3) Quantikine kit, mouse anti-VEGF; (4) Duoset, mouse anti-VEGF. Detection reagent: (1) mouse anti-VEGF (Genetech 4.6.1); (2) rabbit anti-VEGF; (3) mouse anti-VEGF; (4) mouse anti-VEGF. Not surprisingly, each assay reports a different normal range. In our experience the Quantikine kit measures low (with up to a third of samples having undetectable levels) and the Duoset combination measures high, as we reported in the article (648 pg/ml). The other assays report intermediate values.

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References


The important observation in our study is not the absolute VEGF plasma values but the relative differences in VEGF levels between patients and control samples over time, where the sampling issues have been fully appreciated and rigorously controlled to allow clinical interpretation of the results. We have emphasised the prognostic value of plasma VEGF in idiopathic pulmonary fibrosis and have shown a significant positive relationship between the HRCT fibrosis score and the plasma concentration of VEGF. A comparison with acute respiratory distress syndrome (ARDS) is not useful and perpetuates the concept that ARDS equates with chronic idiopathic pulmonary fibrosis, which is not the case.

In relation to the point about quantitating local VEGF concentrations in the lung where the influence of epithelial sflt-1 might be greater, it is to be expected that different assays could lead to a variation in reported VEGF levels for the reasons already discussed.

In conclusion, we have complete confidence in the validity and reproducibility of the VEGF data presented. In a situation of excess VEGF production which could potentially be driving an angiogenic fibrotic response, excess VEGF in order to control the angiogenic fibrotic process might be required to control the VEGF data presented. In a situation of excess VEGF production which could potentially be driving an angiogenic fibrotic process, excess VEGF secretion which could result in proteins with modified carboxy terminal region could lead to the apoptosis of both epithelial and endothelial cells. Clearly, it is a case of restoring homeostasis.

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CARD 15 gene mutations in sarcoidosis
In the last few years substantial progress has been made in unravelling the genetic basis of susceptibility to Crohn’s disease. Three CARD 15 (previously called NOB1) genetic variants, resulting in proteins with modified carboxy terminal regions, have been implicated. 33% of patients with Crohn’s disease carry at least one of these CARD 15 mutations (compared with 1% of healthy controls). 1 Mutations in CARD 15 have also been identified in affected members of families with Blau syndrome.2 This is a rare autosomal dominant disorder, sometimes referred to as familial sarcoidosis, characterised by granuloma formation in joints, skin, and uvea.

Card 15 is a microbial sensing protein involved in innate immunity. It recognises conserved structural components of microorganisms (bacterial muramyl dipeptide, MDP and peptidoglycan, PGN) and is part of the danger signal pattern recognition network which forms the front line of protective immunity. Mutations associated with Crohn’s disease render the molecule insensitive to MDP and interfere with the downstream activation of NF-κB. One potential result of this may be the persistence of inflammation resulting in engagement of other arms of the adaptive immune system and formation of granulomas. Expression of CARD 15 in monocytes (precursors of macrophages and granulomas) further supports its role in granuloma formation. We hypothesised that mutations in CARD 15 may be a unifying defect in the multisytemic granulomatous diseases of Crohn’s disease, sarcoidosis, and Blau syndrome.

To investigate this we recruited a cohort of 29 patients with sarcoidosis from the Oxford Centre for Respiratory Medicine. All had a typical clinical picture of sarcoidosis and either histologically proven disease or characteristic Lofgren’s syndrome (defined as an acute onset of disease with erythema nodosum, joint pains, and bilateral hilar lymph adenopathy). The diagnostic criteria of sarcoidosis were also supported by the characteristic appearance of the lungs on a high resolution computed tomographic (CT) scan in all patients. The definition and diagnosis of sarcoidosis adheres to the statement on sarcoidosis adopted by the joint committees of WASOG/ATS/ERS.4 The patients were first diagnosed between the ages of 25 and 40 years and had been followed up for at least 1 year before recruitment. All were white and one third presented with Lofgren’s syndrome. Written informed consent was obtained for genetic analysis and the study was approved by the local ethics committee.

The entire coding region of CARD 15 (11 exons and flanking intronic sequences) was screened for the presence of mutations. In brief, the CARD 15 gene was amplified from the genomic DNA samples by polymerase chain reaction (PCR) using primers as previously described5 and sequenced on an ABI 377 automated sequencer using a Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer Applied Biosystems, CA, USA). Sequence data were then aligned using the Sequence Navigator analysis software Version 1.0.1 (Perkin Elmer Applied Biosystems) and compared with the known CARD 15 sequence (EMBL accession numberAJ303140).

435 sequence analyses were performed in the 29 patients. We were therefore able to detect new alleles putatively associated with the sarcoidosis phenotype with frequencies as low as 2%. The three mutations associated with Crohn’s disease were specifically examined. The R702W mutation was observed in one patient with sarcoidosis while the G908R and the 1007fs mutations were not found. These results were not different from those reported previously in control populations where the mutations R702W, G908R and the 1007fs were present in 4%, 1%, and 2% of European healthy controls.6 Furthermore, they do not differ from data derived from healthy controls recruited in the UK.7 The codons 334 and 469 reported to be involved in Blau syndrome were also carefully scrutinised but we did not detect any genetic variation in our sarcoidosis patients. No additional mutations were seen within the rest of the coding region of the gene, suggesting that there were no specific alternative CARD 15 mutations associated with sarcoidosis. Schuurman and colleagues8 recently found no correlation between specific CARD 15 polymorphic alleles and patients with sarcoidosis from families with more than one member afflicted by the disease. This study provides evidence that CARD 15 is not associated with non-familial sarcoidosis (in patients with a white ethnic background) and that there are no mutations in any part of the coding region of the CARD 15 gene in the patient cohort.

There is little doubt that there is a genetic predisposition in sarcoidosis, as indicated by the presence of familial clustering, ethnic susceptibility, and recent evidence of an association with HLA-DRB1.9 Furthermore, this susceptibility gene(s) has recently been pointed to a locus near the HLA DR region on chromosome 6. This and the exclusion of the NOB2 locus has focused attention on abnormalities in antigen presentation and cytokine/chemokine receptors as a potential basis for the aetiology of sarcoidosis.

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References

Infected pulmonary sequestration caused by Mycobacterium kansasii

Mycobacterium kansasii is the second most commonly diagnosed nontuberculous mycobacterial associate with lung disease in the United States.1 Diseases are almost always cavitary, involving the upper lobes with fibrosis and infiltrations. Underlying pulmonary diseases associated with M. kansasii infection include pneunomociosis, chronic obstructive lung disease, AIDS and malignancy. To our knowledge, M. kansasii has never been reported in the English literature in the aetiology of infected pulmonary sequestration. We report a case of intralobar sequestration complicated by infection with M. kansasii.

A 33 year old man was previously healthy. An abnormal finding on the chest radiograph had been noted at a routine health check 1.5 years earlier. He had no history of medical illness and no risk factors for HIV infection. No symptoms attributed to his respiratory circulation. Despite the high frequency of infection, few data are available regarding specific infecting organisms. Acute infections due to bacteria such as Staphylococcus and Pseudomonas have been reported, and some chronic infections due to Aspergillus and Nocardia have also been described. Infected pulmonary sequestration due to mycobacteria including M. tuberculosis and M. avium-intracellulare have been reported only rarely. In our case, M. kansasii was the first organism thought to be responsible for the infection of pulmonary sequestration in the absence of other pulmonary or extrapulmonary involvement. It is assumed that the organism reaches the sequestration through Kohn’s pore without causing evident disease in the normal lung parenchyma.

The combination of irononizid, rifampin, and ethambutol was recommended for treatment of M. kansasii pulmonary disease.1 However, surgical removal should be advocated in both asymptomatic and symptomatic cases of pulmonary sequestration and the benefit of a period of prospective antimicrobial treatment in infected pulmonary sequestration has been debated.2 Our patient simply underwent a surgical resection and did not receive any anti-mycobacterial agents.

We have reported the unique occurrence of pulmonary sequestration with M. kansasii infection. Surgical resection allows establishment of the exact diagnosis and immediate removal of the infectious focus, thus preventing complications related to the infection or to the malformation itself.

References
CARD 15 gene mutations in sarcoidosis

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Burkholderia infection and survival in CF

We read with interest the paper by Jones et al. indicating the experience of the Manchester Adult CF Unit in the survival of patients with cystic fibrosis (CF) in the first 5 years following chronic infection with the B cepacia complex (Bcc).

The authors appear to have shown that 31 patients with B cenocepacia had a worse prognosis than Pseudomonas aeruginosa infected patients. Despite the title of the paper, they had insufficient patients chronically infected with B multivorans to draw any similar conclusions. Although these are not new findings, we congratulate them on their attempt to throw light on a difficult topic within the microbiology of CF. However, there are several anomalies in the study that cause concern. Firstly, they state that the 5 year survival in the B cenocepacia group was 66.6%, yet the figure (which incidentally contains data for 7 years) clearly shows the rate was 45% at 5 years and 30%. Also, table 2 states that 19 (of 31) of these patients (61%) died within the study period, a value that fits with neither of the two previous statements. Secondly, although there were significantly more deaths in the B cenocepacia group, these patients apparently did not have increased treatment requirements or diminution in spirometric parameters compared with the matched group. This implies deterioration in respiratory function that was rapid enough to not affect the statistical calculation, but that falls short of the "cepaic" syndrome, which seems unlikely. We wonder whether a different statistical method may have produced more meaningful results. Finally, the authors state in the discussion that the only other studies of outcome of Bcc infection are in CF patients following transplantation. We are surprised that they appear to be unaware of the well conducted study led by Ledson et al from our unit, published in this journal in 2002, which showed the outcome for 37 CF patients chronically infected with B cenocepacia, none of whom were listed for transplantation. This study used a more robust method of statistical analysis to show that B cenocepacia infected patients had an accelerated lung function with a fourfold increased risk of mortality and a trend towards worsening nutrition—results in keeping with those produced by the US CF Foundation.

However, we do agree with the authors that further work needs to be done to assess the effect of infection by other genomovars (including B multivorans) on morbidity and mortality in patients with CF.

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text references

References


Authors’ reply

We thank Drs Ledson and Walshaw for their interest in our recent paper, although we are not in agreement with some of their comments. They feel that there are anomalies for fig 1; they need to inspect it again more carefully. The survival for 1 year (12 months) and 3 years (60 months) is given in the table as 80% and 66%, as is represented in the figure. The timescale on the x-axis in fig 1 is given in months. The data in the figure are not limited to 5 years as it contains survival data for patients throughout the entire study period. For some patients, data were available and are presented over a decade rather than the 7 years suggested by Ledson and Walshaw.

As is clearly stated in the paper, the data for spirometry, body mass index and treatment requirements were, however, limited to 5 years from onset of infection. We collected annual spirometry figures from time of acquisition of infection. We did not demonstrate a significant difference in the decline in FEV1 or FVC between the two groups of patients infected with Burkholderia cenocepacia and Pseudomonas aeruginosa, respectively, but we are unable to exclude the possibility that there may have been a large fall in spirometric parameters in the last few months before death in patients infected with B cenocepacia. If the rate of decline in spirometric data is linear, the use of linear regression—as suggested by Drs Ledson and Walshaw—would not significantly alter the findings. We note that they reported a linear rate of decline in their previous study. We also observe that the study by Ledson et al did not match the patients for spirometry at baseline. The effect of the B cepacia complex (Bcc) on lung function and spirometry is complex and, as mentioned in our discussion, other studies have also shown a decreased survival among patients with Bcc without any demonstrable difference in lung function decline.

Drs Ledson and Walshaw have looked at survival at their own centre. Although a relatively recent paper, they used the redundant term of B cepacia in their title rather than the Burkholderia cepacia complex which is the current nomenclature. It is accepted that infection with the ET12 B cepacia strain confers a clinical disadvantage, and many authors have reported their own experiences of a poor clinical outcome following infection over the past two decades. Our study was the first to compare survival between P aeruginosa infected patients and those with different Bcc genomovars (other than the papers discussed in our article that look at this after transplantation). It presents data to show that adults with cystic fibrosis (CF) infected with some Bcc genomovars have the same outcome as those infected with P aeruginosa. Our study has been supported by another recently published paper from the Belfast CF Centre.

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References


Correction

CARD 15 GENE MUTATIONS IN SARCOIDOSIS

The order of the authors of this letter which appeared on pages 354–355 of the April issue of Thorax was published incorrectly. The correct order is: L-P Ho, F Merlin, K Gaber, R J O Davies, A J McMichael, and J-P Hugot. The publishers apologise for the error.