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. It would have been interesting to know the bronchoalveolar lavage (BAL) fluid levels of VEGF in these patients as Meyer et al and Koyama et al3 have shown reduced BAL fluid VEGF levels in interstitial lung disease. This might simply reflect damage to the alveolar epithelium (a known major source) in this disease or, indeed, VEGF may have an important role in the pathogenesis of interstitial disease. Interestingly, VEGF receptor blockade has been shown to lead to an induction of apoptosis and an in vivo assay of VEGF receptor expression in the lung.4 In addition, it is interesting to speculate on the cellular source of the increased plasma levels of VEGF in the more fibrotic patients. Could the alveolar-capillary membrane damage with leakage of intra-alveolar VEGF which is known to be compartmentalised and hence lower BAL fluid levels as described in the previous studies?5 Or does it represent an inflammatory cell source of systemic VEGF correlating with an inflammatory response that is here associated with a poorer outcome? Or is there some other mechanism? Finally, Koyama et al have shown that smokers also have reduced BAL fluid levels of VEGF and this may be of relevance (if intrapulmonary VEGF is postulated as having a role in this disease), given that the patients with desquamative interstitial pneumonia had all smoked compared with 50% of those with non-specific interstitial pneumonia and only 20% of the controls.

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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.1 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.2 Indeed, HRCT scanning is perhaps the most reliable surrogate for the extent of disease.3 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 BAL fluid 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 intraluminal secretion and the existence of a concentration gradient from air spaces to intravascular spaces.4

Thickett et al 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 ELISA kit. They point out that this range is quoted by the kit manufacturers and is consistent with their own data and, indeed, with other published studies.5 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 purposes from R&D Systems”.6 R&D currently sell these paired reagents under the name “Duoset”.7 Different ELISA formats for VEGF quantitation using recombiant VEGF165 as standard are available. Capture reagent: (1) rabbit polyclonal anti-VEGF (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.8 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.

A number of possibilities exist as to why these assays read differently. It is unlikely to be due to platelet release as suggested.9 The greatest difference in VEGF levels that is detected between paired serum (complete platelet release of VEGF) and standard plasma samples (low platelet VEGF release) is at most only three to four fold. Similarly, the difference in VEGF between paired platelet poor and platelet rich plasma samples is of the same order of magnitude. To demonstrate this one has to spin plasma samples at 2700g for 15 min to prepare platelet poor plasma samples. In our experience it is important to use plasma rather than serum samples to quantitate VEGF as plasma treatment each study sample similarly in terms of centrifugation—whether it be 300g for 12 min, 1000g for 15 min, or 2700g for 15 min. This will minimise variation in the study samples due to the “platelet release effect”.

There are possible explanations for the VEGF immunoassays reading differently in plasma. It may be a combination of at least two effects—the nature of the epitope detected and the presence of other competing ligands in the sample. Different antibodies raised to VEGF will react variably with available epitopes on the ligand. This can be quite striking with monoclonal antibodies to different epitopes of a ligand when they are used as ELISA capture reagents. Antibody A reacts with an epitope on VEGF that is close to or part of the flt-1 receptor binding site and the other antibody B reacts with an epitope well removed from this site, with an identical absolute amount of VEGF in the plasma sample, antibody A would read low or negative and antibody B would read high in relation to the amount of sflt-1 present in the plasma sample. We have in fact shown that the capture antibody used in the Quantikine VEGF ELISA is, indeed, sensitive to the presence of sflt-1.9

In addition to this potential variation in the level of free VEGF and VEGF-sflt-1 complexes in plasma samples, a further confounding species is the amount of placenta growth factor (PLGF). VEGF is a natural homodimer but it does form heterodimers with PLGF and we have detected such complexes.10 Antibodies detecting epitopes that are variably modulated by the binding of PLGF to VEGF will read low or high depending on the PLGF concentration. This focuses on the nature of the R&D Systems monoclonal antibodies to VEGF—one in the Quantikine kit and the other in the Duoset. Following the recognition of a difference in performance of these two assays, we contacted R&D Systems for information concerning the nature of these antibodies. We were interested to know if the same antibody or different antibodies were used and what information was available on their specificity. The response from R&D Systems was that the capture antibodies were different, so the scenario outlined above is a possible explanation.

It is important to appreciate the difficulties in interpreting absolute levels of VEGF in complex media such as plasma. To do this rigorously one ought to quantitate not only free VEGF but also VEGF complexes with sflt-1, sKDR, and PLGF to understand how an increase in one assay may mask an increase in another, and currently this is not possible. It would be simplistic to think that the Quantikine kit values are the “true” VEGF values and the Duoset assay values artefactual. It might simply reflect the fact that the Quantikine values are free VEGF and the Duoset values total VEGF (free VEGF plus VEGF complexes).
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 empha-
sised 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 concentra-
tion 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 slf-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 confi-
dence in the validity and reproducibility of the
VEGF data presented. In a situation of excess VEGF production which could poten-
tially be driving an angiogenic fibrotic
pathology in the lung, we suggest it is entire-
ly appropriate to contemplate antago-
nising excess VEGF in order to control the
disease pathophysiology. Overdosage on
VEGF antagonist is clearly counterproductive
and, as stated, 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.
43% of patients with Crohn’s disease carry at
least one of these CARD 15 mutations (com-
pared with 1% of healthy controls). 1 Mutations in CARD 15 have also been identi-
fied in affected members of families with Blau
syndrome. 2 This is a rare autosomal dominant
condition, 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 microor-
ganisms (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-kB. One potential result of this may be
the persistence of a pathogen, resulting in
granuloma formation. We hypothesised that mutations in CARD 15 may be a unifying
defect in the multisystemic 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 char-
acteristic Loefgren’s syndrome (defined as an
acute onset of disease with erythema nudo-
sum, joint pains, and bilateral hilar lymph-
adenopathy). The diagnosis of sarcoidosis was also supported by the characteristic
appearance of the lungs on a high resolution
tomographic scan (CT) in all patients. The
definition and diagnosis of sarcoidosis adhesion to the statement on sarcoi-
dosis adopted by the joint committees of
WASOG/ATS/ERS. 3 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 Loefgren’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 pre-
viously described 4 and sequenced on an ABI
377 automated sequencer using a Dye
Terminator Cycle Sequencing Ready mix
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 number
AJ303140).

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 exam-
ined. 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
1007fs were present in 4%, 1%, and 2% of
European healthy controls. 4 Furthermore
they do not differ from data derived from
healthy controls recruited in the UK. 4 The
codons 334 and 469 reported to be involved in
Blau syndrome were also carefully scrutinised
but we did not detect any genetic variations
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. Schurmann and
colleagues 5 recently found no correlation
between specific CARD 15 polymorphic
alleles and patients with sarcoidosis from
sarcoidosis, and Blau syndrome.

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. 6 Furthermore
for this susceptibility gene(s) has recurrently
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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Infective pulmonary sequestration caused by *Mycobacterium kansasii*

*Mycobacterium kansasii* is the second most common nontuberculous mycobacteria associated 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 pneumocystis, chronic obstructive lung disease, AIDS and malignancy. To our knowledge, *M. kansasii* has never been reported in the English literature in the aetiology of infective 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 system, such as cough, haemoptysis or dyspnoea, were noted. Constitutional symptoms including malaise, fever, night sweats, anorexia, or weight loss were lacking. Physical examination revealed systolic bruit at the left basal lung. There was neither cyanosis nor digital clubbing. Oxygen saturation by pulse oximetry was 99% while breathing room air. Blood laboratory studies disclosed a white blood cell (WBC) count of 7.4 x 10⁹/l (neutrophils 60.7%, lymphocytes 13.6%), and the level of carcinoembryonic antigen (CEA) was 1.33 ng/ml (reference value <3 ng/ml). Sputum acid-fast stain and mycobacterial cultures were all negative. Bronchoscopic examination showed no endobronchial abnormality. A chest radiograph taken at admission and a computed tomographic scan showed a patchy consolidation over the left basal lung. Magnetic resonance imaging (MRI) and angiography of the chest showed that an aberrant bronchial artery had arisen from the left side of the thoracic aorta and crossed the territory of the consolidation patch, but the venous drainage could not be visualised clearly (fig 1).

The patient underwent lobectomy of the left lower lobe by a thoracoabdominal sternotomy incision. A 14 x 10 cm area of consolidation over the left lower lobe was noted and cystic formation and profuse sputum were found within the lesion. The three feeding arteries from the aorta at the level of the 10th thoracic spine were seen. Microscopically, the parenchyma revealed bronchiolitis and microabscess formation. Granulomatous inflammation was also present, but no organisms were identified by acid fast and Grocott’s methenamine silver (GMS) stains (fig 2). The biopsy tissue was ground and inoculated onto Lowenstein-Jensen slant (BBI, Microbiology Systems, Cockeysville, MD, USA) and cultured at 35°C in a CO₂ incubator. After 19 days of incubation the culture yielded a photochrome which was later identified as *M kansasii* using conventional biochemical methods. The patient had an uneventful postoperative course and was discharged soon after operation. No anti-mycobacterial regimen was administered after the diagnosis was made and no anti-inflammatory drugs remained uneventful at the 3 month follow up.

Pulmonary sequestration is a congenital malformation characterised by the presence of lung tissue with abnormal or absent communication with the bronchial tree and arterial blood supply from the systemic 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 isoniazid, rifampicin, 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 procoagulant anticoagulation treatment in infected pulmonary sequestration has been debated.² Our patient simply underwent a surgical resection and did not receive any anti-mycobacterial agent.

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.

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**References**


![Figure 1](https://example.com/image1)

**Figure 1** Magnetic resonance imaging (MRI) and angiography of chest showing the aberrant bronchial artery (arrow) arising from the thoracic aorta crossing the consolidation.

![Figure 2](https://example.com/image2)

**Figure 2** Microscopic section of the lung parenchyma showing microabscesses and granulomatous inflammation (arrow).
VEGF in idiopathic ILD

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Thorax 2005 60: 353-354
doi: 10.1136/thx.2004.033159

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