Appendix 1

Methods

Identification of cases

Potential incident cases of idiopathic pulmonary fibrosis (IPF) were identified from five teaching general hospitals and eight district general hospitals in the Greater Trent Region and Wales (see Figure 1). We defined incident cases as someone newly diagnosed with IPF (ATS/ERS criteria for diagnosis)[1] by a respiratory physician in the six months prior to the start of the study and throughout the recruitment period. Cases were required to have a clinical history of cough or dyspnoea and either a high resolution computed tomography (HRCT) scan which was suggestive of usual interstitial pneumonia (UIP) or a histological confirmation of UIP. Potential cases with co-existing connective tissue disease and/or a clinical picture suggestive of extrinsic allergic alveolitis (EAA) were excluded from the study population. HRCT scans for all potential cases were reviewed by two experienced thoracic radiologists (KP and MK) to confirm the diagnosis of IPF and to stratify cases to diagnoses of definite or probable UIP, fibrotic non-specific interstitial pneumonitis (NSIP) or unclassifiable fibrotic lung disease (see Table 1, Images 1a-l,2a-h,3a-h,4a-f;online supplement). We have previously shown good inter-observer agreement between these two thoracic radiologists (K=0.67).[2]

Data collection

All participants then had a venous blood sample taken using BD Vacutainer® tubes containing either 0.109 M sodium citrate, K_2 EDTA or clot activator and gel for serum separation. As only incident cases were recruited into the study, all blood samples were taken soon after the diagnosis of IPF was made and when cases were clinically stable (median time from diagnosis to venous sampling of six months).

Laboratory analysis

Sodium citrate samples were centrifuged twice for 10 minutes each time at 2000 x g and serum samples were centrifuged for 10 minutes at 1300 x g. Plasma and serum were stored at -80°C while whole blood samples were stored in EDTA bottles at -20°C until laboratory analysis. As some of the coagulation proteases act as acute phase response proteins, we also measured highly sensitive C reactive protein (hsCRP) as a potential confounder.

Statistical analysis

All cases and controls were grouped into five year age bands over the age of 65 years. Smoking habit was defined as current, ex and never smokers, and hsCRP levels were grouped into three categories previously used to define cardiovascular risk.[3] We used standard clinical laboratory baseline values for the coagulation proteases measured with the exception of antithrombin III and Factor VIII levels. We defined antithrombin III deficiency as levels below two standard deviations from the mean value of the control group and Factor VIII levels above 165 IU/dL were considered elevated. A higher baseline value for Factor VIII was used instead of the conventional value of 150 IU/dL because it has been demonstrated that Factor VIII levels increase with age[4] and Factor VIII levels of individuals in this age groups is unknown.

Figure 1: Flow chart depicting recruitment of incident cases of IPF and general population controls

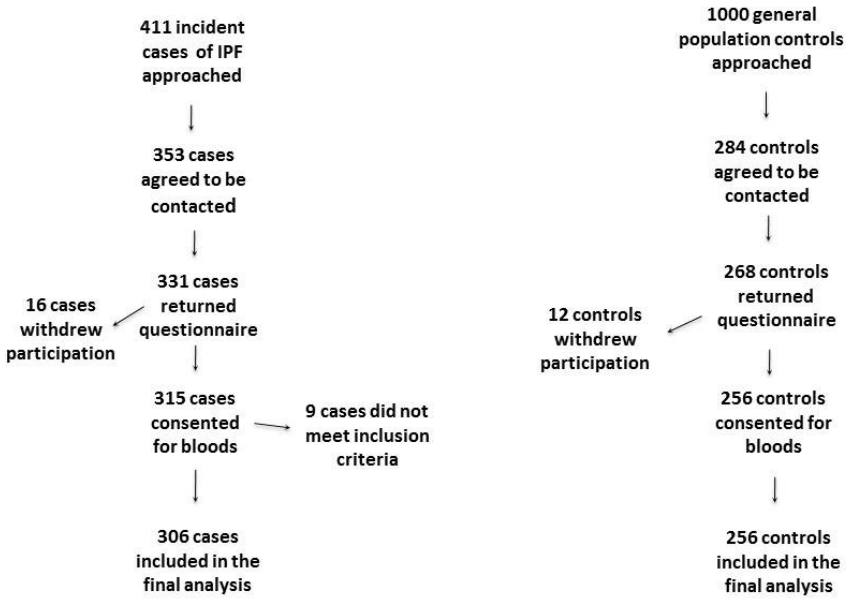

Table 1: High resolution computed tomography (HRCT) diagnostic criteria for usual interstitial pneumonia (UIP) [1]

Definite UIP pattern	Probable UIP pattern
Sub pleural, basal predominance□ Reticular abnormality□ Honeycombing with or without traction bronchiectasis□ Absence of features listed as inconsistent with UIP pattern	Sub pleural, basal predominance□ Reticular abnormality□ Absence of features listed as inconsistent with UIP pattern

References:

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2. Navaratnam V, Pointon K, Kumaran M, et al. Are Radiological Appearances In Pulmonary Fibrosis Dependent On The Experience Of The Reporting Radiologist And Can They Predict Survival? American Journal of Respiratory and Critical Care Medicine. 2012 May 1, 2012;185(1 MeetingAbstracts):A4380.
3. Pearson TA, Mensah GA, Alexander RW, et al. Markers of Inflammation and Cardiovascular Disease. Circulation. 2003 January 28, 2003;107(3):499-511.
4. Rumley A, Emberson JR, Wannamethee SG, et al. Effects of older age on fibrin D-dimer, C-reactive protein, and other hemostatic and inflammatory variables in men aged 60-79 years. J Thromb Haemost. [Research Support, Non-U.S. Gov't]. 2006 May;4(5):982-7.