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Immunoglobulin A in serum: an old acquaintance as a new prognostic biomarker in idiopathic pulmonary fibrosis

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Summary

Immunoglobulin (Ig)A is an important immunoglobulin in mucosal immunity and protects the lungs against invading pathogens. The production of IgA is regulated by transforming growth factor (TGF)-β, a versatile cytokine and key player in the pathogenesis of pulmonary fibrosis. TGF-β is up-regulated in patients with idiopathic pulmonary fibrosis (IPF), but difficult to use as a biomarker. The aim of this study was to evaluate the prognostic value of IgA in serum in patients with IPF. We examined IgA levels at time of diagnosis in 86 patients diagnosed with IPF. Mean serum IgA level in IPF is 3·22 g/l and regression analyses showed a significant association with mortality (hazard ratio = 1·445, P = 0·002). A significantly worse survival was found in patients with IgA serum levels > 2·85 g/l compared to patients with lower IgA serum levels (P = 0·003). These findings were confirmed in a duplication cohort. In conclusion, the level of IgA in blood is a promising prognostic marker in IPF and can be implemented easily in the hospital setting. Future studies are warranted to investigate if repeated measurements of serum IgA can further improve the performance of serum IgA as a prognostic marker.

Keywords: fibroblasts, human, lung, transplantation

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive parenchymal lung disease with a poor prognosis of 3–4 years after diagnosis. The pathogenesis of IPF remains unclear, although several environmental factors may contribute – for example, cigarette smoking and chronic viral infections [1]. To protect the lungs against pathogens invading the mucosa, immunoglobulin (Ig)A is present in the mucous secretions of the lungs [2]. Human IgA can be found in various mucous secretions and produced locally by plasma cells. IgA also circulates in blood, and although the overall function in the circulation is not well understood, it is thought that its main function is to clear the circulation of immune complexes by phagocytosis [3]. The majority of IgA is produced locally by plasma cells. *In-vivo* studies in transforming growth factor (TGF)-β knock-out mice demonstrated that TGF-β plays an important role in IgA production [4–6]. TGF-β is a multi-functional cytokine that is involved in multiple signalling processes and has profound regulatory effects on many developmental and physiological processes, such as embryogenesis, cell growth, immune functions and wound healing [7,8]. In the lungs, secretion and activation of latent TGF-β is caused by abnormally activated alveolar epithelial cells (AECs) in IPF. Activated TGF-β is responsible for the differentiation of fibroblasts to myofibroblasts and the formation of the typical fibroblast foci in IPF [1]. In the different mechanisms proposed to be involved in the pathogenesis of IPF, all identified TGF-β as a key player in the development of IPF [1,9–11].

Activated TGF-β is up-regulated by AECs in IPF [12–14], and we hypothesized that an increase in activated TGF-β is reflected by an increase in IgA in blood. Therefore, IgA serum could be a practical and well-needed biomarker for the prognosis of IPF, as IgA serum measurements are easily accessible. To study the potential role of serum IgA as a prognostic biomarker in IPF we examined a retrospective cohort of IPF patients and reproduced our findings in a prospective duplication cohort.

Material and methods

Study patients

The initial study cohort consisted of 86 patients with IPF diagnosed before 2007. Diagnoses were revised and

Table 1. Baseline characteristics of the initial and duplication cohort

	Initial cohort ($n = 86$)	Duplication cohort ($n = 83$)	Significance
Gender (%)			
Male	67 (78)	73 (88)	n.s.
Female	19 (22)	10 (12)	n.s.
Age (s.d.)	59·4 (12·9)	62·9 (8·7)	n.s.
Smoking status (%)			
Yes	9 (10)	3 (4)	n.s.
Never	24 (28)	20 (24)	n.s.
Former	42 (49)	58 (70)	n.s.
Unknown	11 (13)	2 (2)	n.s.
Pulmonary function tests (s.d.)			
DLco% of predicted	45·1 (16·3)	46·6 (15·1)	n.s.
FVC% of predicted*	72·6 (23·5)	82·8 (24·3)	n.s.
TLC% of predicted	66·1 (18·9)	68·4 (14·4)	n.s.

DLco = diffusing capacity for carbon monoxide; FVC = forced vital capacity; TLC = total vital capacity; s.d. = standard deviation; n.s. = not significant. Statistically significant difference between both groups at the 0·05 alpha level.

patients were included when the criteria set by the American Thoracic Society (ATS), The European Respiratory Society (ERS), the Japanese Respiratory Society (JRS) and the Latin American Thoracic Association (ALAT) (ATS/ERS/JRS/ALAT) were met [10]. We recruited a prospective duplication cohort of 83 IPF patients all diagnosed after 2007 until July 2013. Time of diagnosis was defined as the date of first multi-disciplinary meeting between pulmonologist, radiologist and pathologist.

Patient demographics, smoking status and lung function were determined at diagnosis.

One patient was excluded because of an IgA deficiency and hypogammaglobulinaemia due to a common variable immune deficiency (CVID). The research was conducted using appropriate ethical guidelines and was approved by the Medical Ethical Committee VCMO (Verenigde Commissies Mensgebonden Onderzoek) of St Antonius Hospital in Nieuwegein. All participants gave written informed consent.

Evaluation of serum immunoglobulin IgA, IgG and IgM

Serum samples were collected at the time of diagnosis. The majority of the serum samples were collected routinely for monitoring purposes, and in a few cases immunoglobulin measurements were performed on stored serum samples. Immunoglobulin measurements were performed by the Immage® 800 Beckman Turbid meter (Woerden, the Netherlands).

Statistical analysis

Differences in demographic and clinical characteristics between both cohorts were compared with the use of the independent-samples *t*-test for continuous variables and the χ^2 or Fisher's exact tests for categorical variables, as appropriate. There are no adult age-specific and gender-specific values for serum IgA concentrations. Exploratory analysis demonstrated a linear relationship between increasing IgA level and risk of mortality. The Cox proportional hazard regression model was used for the primary analysis to evaluate the associations of serum immunoglobulines with mortality. Survival estimates were calculated with the Kaplan–Meier method. For survival calculation the patients were censored at the time of transplantation or when patients were still alive at the end of the study. The optimal cut-off point of IgA between the two survival groups was calculated with a receiver operating characteristic (ROC) analysis. Comparison of survival in different groups was performed with the log-rank test. Cox regression analysis was used to test the effect on the variables on overall survival. All tests were evaluated at the 0·05 alpha level and *P*-values were two-sided. Analyses were performed using SPSS version 21·0 (SPSS Inc., New York, USA).

Results

The initial cohort included 86 IPF patients, and during a follow-up of 48 months 69 patients died and 13 patients underwent lung transplantation. The duplication cohort consisted of 83 patients, and during a follow-up of 48 months 43 patients died and two patients underwent lung transplantation. Demographic and clinical characteristics of both cohorts are shown in Table 1. Subgroup analyses demonstrated no significant differences in IgA levels between patients who were current or former smokers and non-smokers.

The mean serum IgA level was 3·22 g/l (range = 0·9–6·8 g/l). No statistically significant associations were found between serum IgA levels and baseline demographic and clinical variables, except for serum IgA levels and DLCO% of predicted, which showed a moderate Spearmans's correlation ($r_s = -0{\cdot}5$, $P < 0{\cdot}001$). The immunoglobulin levels in the duplication cohort were not significantly different compared to the initial cohort; see Fig. 1.

Serum IgA levels associated with mortality

Univariate regression analyses revealed that serum IgA, but not IgG and IgM serum levels, was associated with early mortality [hazard ratio (HR) = 1·445, confidence interval (CI) = 95% 1·14–1·83, $P = 0{\cdot}002$; Table 3]. Regression analysis of both cohorts together showed a significant correlation between IgA in serum and long-term survival (HR = 1·424, CI = 95% 1·216–1·667, $P < 0{\cdot}001$). Multivariate regression analysis correcting for pulmonary function tests showed that serum IgA levels remain predictive of survival (HR = 1·576, CI = 95% 1·224–2·029, $P < 0{\cdot}001$).

Table 2. Mean serum immunoglobulin levels of both idiopathic pulmonary fibrosis (IPF) cohorts

	n	Mean g/l (s.d.)	Range
Cohort 1			
IgA	86	3·22 (1·38)	0·9–6·8
IgG	85	12·07 (3·16)	5·7–19·4
IgM	85	1·10 (0·58)	0·3–3·5
Cohort 2			
IgA	83	3·61(1·45)	0·7–6·9
IgG	83	12·42 (3·54)	4·1–22·0
IgM	82	1·07 (0·79)	0·2–4·1

Ig = immunoglobulin; s.d. = standard deviation.

The median overall survival of the initial cohort is 4·4 years and the median survival of the duplication cohort is 3·4 years. None of the patients were lost to follow-up. After dividing the patients into groups according to the cause of death, mean IgA serum levels were calculated: progressive lung fibrosis mean IgA 2·71 g/l; infectious complications mean IgA 3·89 g/l; and acute exacerbation (AE) of IPF 4·05 g/l. The highest levels were found in patients who died after AE or infections and the lowest levels in progressive lung fibrosis [infectious complications *versus* progressive lung fibrosis, $P = 0{\cdot}045$; AE *versus* progressive lung fibrosis, $P = 0{\cdot}038$; infectious complication *versus* AE = not significant (n.s.)]. The cut-off value defining whether a patient is at risk of dying within the first years after diagnosis with highest specificity as well as sensitivity was found at approximately 2·85 g/l [ROC area under the curve (AUC) = 0·71, sensitivity 0.79 and specificity 0·43]. This cut-off value was used for a Kaplan–Meier analyses, as shown in Fig. 2.

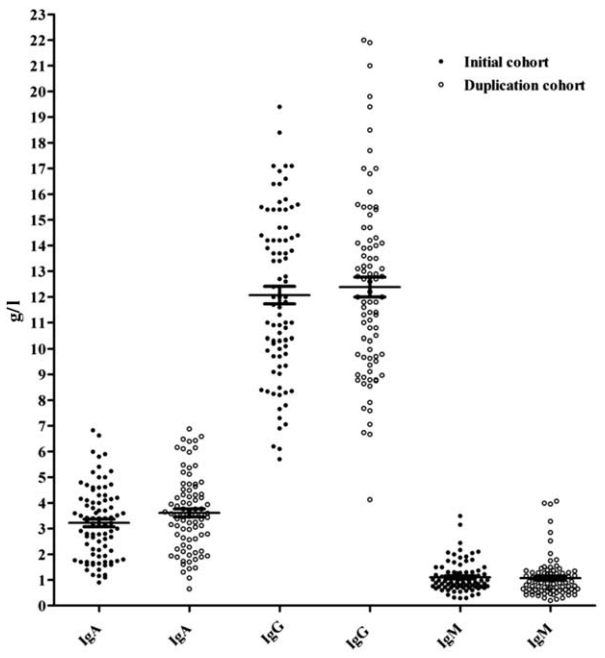

Fig. 1. Shows a comparison of immunoglobulin levels of both cohorts. There are no significant differences between the cohorts [immunoglobulin (Ig)A: $P = 0{\cdot}08$; IgG: $P = 0{\cdot}5$; IgM: $P = 0{\cdot}8$].

Discussion

The results of this study indicate that the IgA level in serum is a promising prognostic biomarker for IPF, in which high IgA levels indicate a worse prognosis.

The search for a biomarker to diagnose and follow-up fibrotic diseases continues, and although many possible prognostic biomarkers have been studied in IPF, to our knowledge this is the first study that investigated serum IgA as a potential biomarker for IPF [15].

Normal IgA levels in serum range from 0·7 to 4 g/l. Most of the serum IgA levels in both of our cohorts varied between normal and slightly elevated levels. Moreover, the established cut-off value of 2·85 g/l lies within the normal range.

Former studies have demonstrated that both bacterial and viral infections cause acute exacerbations in IPF and a higher risk of mortality [16,17]. Furthermore, increased numbers of immunoglobulin-secreting cells in bronchiolar lavage fluid and blood of patients with IPF have been reported [18]. However, it is not clear if the increase of immunoglobulin-secreting cells is caused by the pathogenic process or may be secondary to subclinical triggers such as infection, smoking or air pollution. Recent study demonstrated an increased bacterial load in IPF patients at the time of diagnosis, and that this was associated with rapidly progressive IPF and increased risk of mortality [19]. Analysis of IgA serum levels in different causes of death in our cohort demonstrated higher IgA serum levels in patients who died after an acute exacerbation of IPF or infectious complications, which suggests that levels of IgA are associated with an external cause of death. Further investigation on this topic is warranted.

Production of IgA is regulated by multiple molecules, including TGF-β [20,21]. TGF-β is required for the development of plasma cells secreting all secondary isotypes. One of the best-characterized effects of TGF-β is its ability to stimulate isotype-switching to IgA [22].The role of TGF-β1 in mucosal and systemic IgA production has been studied further in TGF-β1 knock-out mice, and it has been demonstrated that these mice had significantly lower levels of IgA in both blood and mucosal secretions [23]. TGF-β has been characterized as a key profibrotic molecule and is involved in tissue repair processes. TGF-β is up-regulated during wound healing, and it is thought that persistent activation of TGF-β signalling causes fibrotic diseases, such as lung, liver and kidney fibrosis. In line with this observation, TGF-β levels were found to be increased in the lungs of IPF patients [13,24]. Due to the relation between TGF-β and IgA this would – in theory – mean that IgA could be a marker for fibrosis in general. Studies in other organ fibrosis are required to investigate this.

Table 3. Correlation between immunoglobulin in blood serum and survival after diagnosis in idiopathic pulmonary fibrosis (IPF)

Factor	Survival after diagnosis (HR and 95% CI)	*P*-value
Initial cohort		
IgA	1·445 (1·142–1·829)	0·002*
IgG	1·087 (0·976–1·210)	0·129
IgM	1·161 (0·659–2·043)	0·606
Duplication cohort		
IgA	1·360 (1·099–1·683)	0·005*
IgG	1·066 (0·988–1·149)	0·098
IgM	0·891 (0·601–1·320)	0·564

HR: hazard ratio; CI: confidence interval; Ig = immunoglobulin.

TGF-β has a dual role in immune homeostasis; it inhibits T helper type 1 (Th1) cells, Th2 cells and cytotoxic lymphocytes (CTL), whereas it induces differentiation of regulatory T cells and Th17 cells. Furthermore, together with IL-10 and IL-21, TGF-β induces CD40-activated B cells to switch to IgA^+ B cells [20,25].

At first glance, determination of TGF-β in blood would be a logical step in diagnosing and following disease progression in fibrotic diseases such as IPF. However, there is controversy about measuring functional TGF-β and the determination of TGF-β concentrations in blood. Different ranges for the concentrations of TGF-β in blood have been described, and several different recommendations for pre-analytical sample handling can be found [26]. It has been suggested that serum measurements are not useful due to the abundance of TGF-β in platelets, and therefore should be corrected by simultaneous measurement of markers of platelet degranulation [27]. However, others stated that direct measurements could provide a reliable estimate of active and total TGF-β in plasma [28]. In contrast to many other promising biomarkers, IgA assays are part of everyday routine in most clinical laboratories worldwide, with well-known standard values, and the analysis is run on a daily basis.

Prognostic biomarkers for IPF are needed urgently to identify the individual clinical course and to predict prognosis. IPF patients have a poor prognosis and different patterns of survival have been identified [1]. Identification of these different survival patterns is important, because the only medical option to prolong life in these patients is lung transplantation, and early referral of suitable patients can be potentially life-saving [29]. Assessment for lung transplantation is advisable when patients have a 2–3-year predicted survival or less [30]. Lung function and lung function decline seems to be a good marker for early mortality; however, biomarkers such as IgA might capture disease activity more effectively [31]. Future studies could show if repeated measurements of serum IgA level provide a more specific marker of mortality risk and disease severity for long-term follow-up than pulmonary function tests alone.

The strengths of the current study are its well-characterized patient population; classification of patients according to the most recent consensus criteria; the length and completeness of the follow-up; and confirmation of our findings in a duplication cohort.

However, a limitation should also be considered when interpreting our results. Despite no major significant differences in baseline clinical characteristics, the duplication cohort was derived from a different time-span, and awareness of IPF has increased during the last few years.

In conclusion, we have demonstrated that serum IgA level at the time of diagnosis is a predictor of survival in IPF. Increased serum levels of IgA may identify more 'fibrotic active' disease that increases the risk of death in

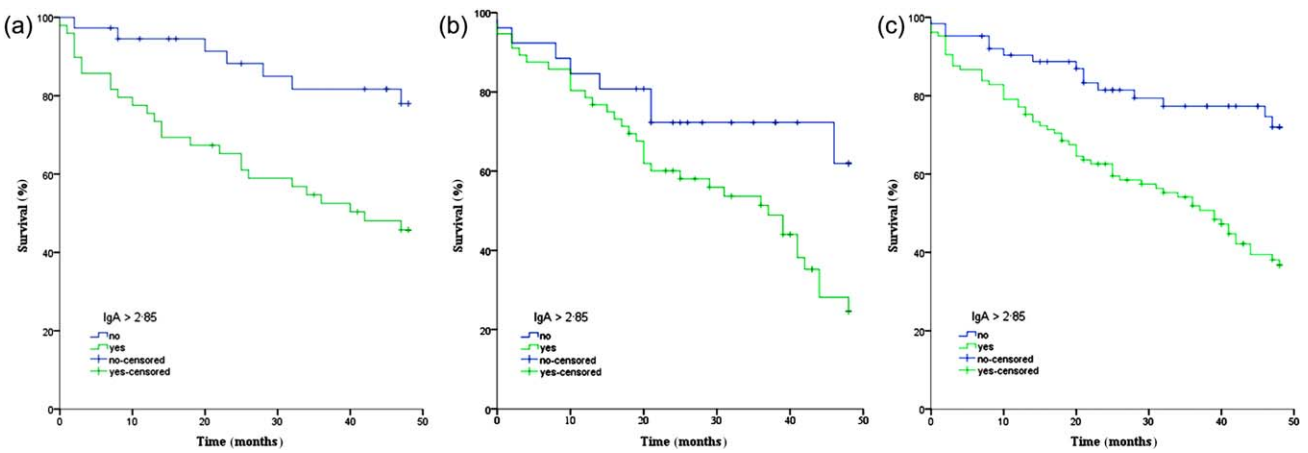

Fig. 2. (a) Demonstrates survival of 86 idiopathic pulmonary fibrosis (IPF) patients (initial cohort). Significant survival difference between patients with immunoglobulin (Ig)A > 2·85 g/l compared to patients with IgA < 2·85 g/l at time of diagnosis ($P = 0·003$). (b) Shows the survival of 83 IPF patients (duplication cohort) and also demonstrates a significant difference in survival between patients with IgA > 2·85 g/l compared to patients with IgA < 2·85 g/l at time of diagnosis ($P = 0·03$). (c) Shows difference in survival of both cohorts together ($n = 168$; $P < 0·001$).

the following year and was not predicted by other baseline, non-invasive clinical predictors, such as pulmonary function tests. These results, along with the broad availability to measure serum IgA levels in many hospitals, makes it easy to use IgA levels for prognostic purposes in IPF. Moreover, IgA level might be an interesting parameter for determination timing of referral for lung transplantation.

Disclosure

The authors have no conflict of interest to declare.

Author contributions

L. K. and C. M. designed the study, H. K., J. G. and H. V. reviewed the paper and L. K. wrote the paper.

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