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Diagnostics

Impact of age on the diagnostic yield of four different biomarkers of tuberculous pleural effusion

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ABSTRACT

The diagnostic value of pleural fluid biomarkers in tuberculous pleurisy (TP) is firmly established. However, it is less clear whether patients' age affects the diagnostic accuracy of TP biomarkers. The aim of the study was to assess the impact of age, on the predictive value of ADA, IFN-γ, IP-10 and Fas ligand in patients with pleural effusion.

The study included 222 patients, median age 64.5 (54–77) years, 58.6% men, with pleural effusion: TPE (60 patients; 27.0%), malignant PE (90 patients; 40.5%), parapneumonic effusion/pleural empyema (35 patients; 15.8%), pleural transudate (30 patients, 13.5%) and other causes of PE (7 patients; 3.2%). The odds ratio for the diagnosis of TPE significantly decreased with increasing age (OR = 0.62/10 years) and significantly increased with increasing level of all evaluated pleural fluid biomarkers. Age affected the diagnostic accuracy of ADA with a trend towards reduction in OR for TPE in older patients (P = 0.077, 95% CI 0.59–1.03).

Younger age and high pleural fluid ADA level are associated with very high probability of TP. This probability significantly decreases not only with decreasing pleural fluid ADA, but also with increasing age. Patient's age does not affect the diagnostic yield of pleural fluid IFN-γ, IP-10 and sFas.

1. Introduction

Tuberculous pleurisy (TP) is usually ranked as the first or second most common manifestation of extrapulmonary tuberculosis [1]. However, the relative contribution of patients with TP is highly variable. In regions with low and intermediate tuberculosis (TB) incidence, these patients usually account for 4–5% of all TB cases [1–3], while in low income and high TB incidence countries the percentage of patients with TP might be as high as 22.3% or even 56 and 68.8% [4–6]. It should also be noted, that even in countries with low or intermediate TB incidence, there is a significant variability between the relative incidence of TP in different country regions. In Poland, the relative incidence of TP in 2015 ranged between 2.9% and 9.9% in Lubuskie and Mazowieckie voivodship, respectively [7]. As there are no significant differences in demographic structure of populations living in these regions, one of the possible explanation for the above variability might be the quality of health care, in particular, the availability and the use of sensitive methods to diagnose tuberculous pleural effusion (TPE). This is critically important in the context of the known limitations of microbiological tests to prove pleural disease related to *Mycobacterium tuberculosis* infection. These limitations are well reflected by the results of two Spanish studies which showed that the sensitivity of acid-fast smear and the cultures on solid media for pleural fluid samples were 6% and 36.3%, respectively [8] and 8% and 41%, respectively [9]. Better results were found for nucleic acid amplification tests (NAATs), but the sensitivity of this method is highly variable (37–71%) and still too low to recommend the use of NAATs in routine work-up of patients suspected of TPE [2,10,11]. The limited diagnostic capacity of NAATs is probably related to the low organism burden in pleural fluid [3].

Low and variable diagnostic sensitivity of the above microbiological tests encouraged the numerous studies on different biomarkers of TPE. As a result, several sensitive and specific biomarkers of TP have been identified with adenosine deaminase (ADA) and interferon-γ (IFN-γ) gaining the widest acceptance. The differences in the diagnostic yield of the same biomarker reported by different authors could probably be associated with a variety of factors including demographic characteristics of the study group, time point of pleural fluid collection, methods of measurement and others. There are numerous data showing the

Abbreviations: ADA, deaminase adenosine; IFN-γ, interferon gamma; IP-10, interferon gamma-induced protein 10; MPE, malignant pleural effusion; PPE/PE, parapneumonic pleural effusion/pleural empyema; sFasL, soluble Fas Ligand; TP, tuberculous pleurisy; TPE, tuberculous pleural effusion

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impact of senescence on the immune system. These include decline in the production of fresh naïve T cells, more restricted T cell receptor (TCR) repertoire, reduced cognate helper function and weak activation of T cells [12–14]. Aging also affects macrophage number and functions, e.g. toll-like receptor signalling, polarisation, phagocytosis, and wound repair [15,16]. As a result, age-related impairments in macrophage function are likely to have important consequences for the health of the older population. Given the above, age-related changes in local ADA activity and IFN-γ level may be expected and this led as to the hypothesis that age-related impairments in CD4(+) cell and macrophage function may have important diagnostic consequences in patients with tuberculous pleurisy. Lee et al. found that patients with TPE and low ADA levels were significantly older than those with high ADA levels. A multivariate analysis revealed that older age was an independent predictor of TP in patients with a low pleural fluid ADA level (odds ratio = 1.053, p = 0.002) [17]. Our previous study showed that ADA, IFN-γ, IP10 and sFasL are sensitive and specific biomarkers of TP, with diagnostic accuracy measured as AUC higher than 0.9 [18]. This was in contrast to the other 6 evaluated biomarkers which had a significantly lower diagnostic yield. The aim of the current study was to assess the relationship between patients' age and the diagnostic accuracy of ADA and three other markers of TP, and in particular, to measure the impact of age - as an independent covariate - on the prediction of TPE in patients with pleural effusion.

2. Material and methods

2.1. Study design

The study was a subset of an investigation project on the diagnostic yield of pleural fluid biomarkers in patients with different pleural diseases. A unified diagnostic approach to patients with pleural effusion and an electronic database that included data on all patients with pleural effusion treated in our institution were developed as a part of that project. The current analysis of the relationship between the patient age and the diagnostic yield of four pleural fluid biomarkers of TPE (ADA, IFN-γ, IP-10 and Fas ligand) was based on data from 253 patients with pleural effusion treated between January 2012 and June 2016. The protocol of the study was approved by the Institutional Review Board of the Medical University of Warsaw, Poland.

2.2. Diagnostic work-up of patients with pleural effusion

Patients with pleural effusion underwent clinical examination which included signs and symptoms and medical history, basic blood laboratory tests, ECG and chest radiograph. In nearly all patients diagnostic thoracentesis and pleural fluid analysis (chemistry, cytology, including total and differential cell count and microbiology) were performed. Pleural fluid samples for further evaluation of biomarkers were frozen and adequately labeled. Effusions were classified as transudates or exudates based on Light's criteria. Additional diagnostic procedures, including blood tests, echocardiography, thorax and abdominal CT scan, abdominal ultrasound, bronchoscopy, mammography and/or breast ultrasound, pleural biopsy or thoracoscopy were at the discretion of the attending physician. A general algorithm used to diagnose patients with pleural effusion in the frame of the project is presented in Fig. S1 enclosed as supplementary material.

2.3. Diagnostic criteria for specific causes of pleural effusion

Only patients with proven TPE were included in the study. The following criteria had to be met to be enrolled: (1) positive culture for *M. tuberculosis* in pleural fluid or pleural biopsy, or (2) positive smear of pleural fluid and positive result of NAAT for *M. tuberculosis complex*, or (3) caseating granulomas in pleural biopsy or positive microbiological results of respiratory samples (sputum, bronchial washing, BALF) with exclusion of alternative causes of pleural effusion and resolution of effusion after antituberculous therapy.

The diagnostic criteria of malignant pleural effusion (MPE), parapneumonic effusion and pleural empyema (PPE/PE), heart failure, pulmonary embolism, hepatic hydrothorax, nephrotic syndrome were consistent with those used in earlier studies [18,19].

2.4. Measurement of TB biomarkers in pleural fluid

Pleural fluid samples for subsequent measurements of biomarker levels (average pleural fluid volume 100 mL) were collected during the first or second diagnostic thoracentesis. The samples were centrifuged at 2000 rpm for 10 min and the supernatant was frozen at −70 °C. Activity of ADA and IFN-γ, IP10 and sFasL concentrations were later measured in thawed pleural fluid samples. ADA activity was determined with colorimetric method by Giusti [20], while the concentrations of IFN-γ, IP10 and sFasL were measured with respective ELISA kits (R&D System, Minneapolis, MN) according to the manufacturer's recommendations. When the level of biomarker was lower or higher than the lowest and the highest detection limit of the kit, a numerical value of the lowest or the highest detection limit was adopted for further calculations.

2.5. Statistical analysis

Data are presented as median and interquartile range (IQR). D'Agostino-Pearson test was used to assess normality of data distribution. Differences between continuous variables were tested using non-parametric Kruskal-Wallis or Mann-Whitney *U* test. The diagnostic performance of a test to discriminate between TPE and non-TPE was evaluated using Receiver Operating Characteristic (ROC) curve analysis which included the calculation of the area under the curve (AUC) and 95% confidence intervals (95% CI) and the odds ratio with 95% CI by means of logistic regression. Probability of TPE diagnosis was quantified as a number between 0 and 100. Prediction of TPE was calculated from the linear logistic regression model. Fitted values from logistic regression model were used as a diagnostic test. All P values were 2-tailed and $P < 0.05$ was considered statistically significant. Statistical analysis was performed with Statistica 13.1 (StatSoft, Tulsa, USA), MedCalc 18.0 (MedCalc Software, Ostend, Belgium) software packages and SAS System 9.4 (SAS Institute Inc., Cary, USA).

3. Results

Two hundred and fifty three patients with pleural effusion were initially selected for analysis. In 8 patients thoracentesis was not performed and in 23 patients the cause of pleural effusion was not established despite an extensive diagnostic work-up. These patients were excluded from the final analysis. Thus, the study group comprised 222 patients with unequivocally diagnosed cause of pleural effusion; 130 (58.6%) men and 92 (41.4%) women; median age 64.5 (IQR 54–77) years. There were 60 patients with TPE (27.0%), 90 patients with MPE (40.5%), 35 patients with PPE/PE (15.8%), 30 patients with pleural transudates (13.5%) and 7 with other causes of pleural effusions (3.2%)

The comparative characteristics of patients with different underlying causes of pleural effusion in terms of their age and the levels of four evaluated pleural fluid biomarkers are shown in Table 1. Multiple comparisons show that patients with TPE were significantly younger than patients with MPE and patients with pleural transudates ($P = 0.0004$ and $P < 0.0001$, respectively), and that the levels of all biomarkers were significantly higher in TPE compared to MPE, PPE/PE, transudates and other effusions.

Analysis in the entire group revealed weak but significant negative correlations between the levels of ADA, IFN-γ, IP-10, sFasL and patients' age. However, in a subanalysis which included only patients with TPE, the correlations between age and biomarker levels were not significant

Table 1
Age and pleural fluid biomarker levels in patients with different underlying diseases. Data are presented as median an IQR; ADA – adenosine deaminase. IFN-γ - interferon gamma, IP-10 – interferon gamma-induced protein 10, sFasL – soluble Fas ligand, TPE – tuberculous pleural effusion, MPE – malignant pleural effusion, PPE/PE – parapneumonic effusion/pleural empyema.

	All patients	TPE	MPE	PPE/PE	Transudates	Other	P
Age (years)	64.5 (54.0–77.0)	53.5 (35.0–71.5)	69.0 (60.0–76.0)	60.0 (50.0–71.0)	78.0 (58.0–83.0)	61.0 (51.0–72.0)	< 0.0001
ADA (U/L)	10.5 (1.5–44)	57.8 (42.9–92.0)	7.4 (2.1–14.0)	12.8 (4.7–49.0)	0.0 (0.0–0.0)	2.3 (0.0–35.6)	< 0.0001
IFN-γ (pg/mL)	8.0 (8.0–33.8)	734.8 (311.1–1000.0)	8.0 (8.0–8.0)	8.0 (8.0–8.0)	8.0 (8.0–8.0)	8.0 (8.0–8.0)	< 0.0001
IP-10 (pg/mL)	2319.0 (593.5–14581.7)	31660.0 21570–34399.0)	1910.0 (606–4119.0)	1127.7 (212.0–3400.0)	879.7 (236.0–1930.8)	499.8 (0.0–1026.0)	< 0.0001
sFas L (pg/mL)	40.9 (18.9–79.9)	86.0 (64.1–133.2)	19.6 (13.7–31.9)	22.3 (16.2–32.2)	NA	NA	< 0.0001

Table 2
Correlations between age and pleural fluid biomarker levels in the entire group and in patients with TPE only. ADA – adenosine deaminase. IFN-γ - interferon gamma, IP-10 – interferon gamma-induced protein 10, sFasL – soluble Fas ligand, TPE – tuberculous pleural effusion.

		Entire group (n = 222)		TPE group (n = 60)	
		R Spearman	P	R Spearman	P
Age and	ADA	−0.23	< **0.001**	+0.04	0.78
	IFN-γ	−0.27	< **0.001**	−0.05	0.76
	IP-10	−0.19	**0.010**	−0.26	0.09
	sFasL	−0.34	**0.001**	−0.15	0.37

(Table 2). Based on two previous reports on the relationship between patients' age and the diagnostic accuracy of pleural fluid ADA, the optimal cut-off values and the diagnostic accuracy of ADA, IFN-γ, IP-10 and sFasL were tested in different age groups: < 45 years old or ≥45 years old and ≤55-years old or > 55 years old [19,20]. In patients aged ≥45 years, the cut-off value was lower for ADA and higher for IFN-γ, while the AUCs for these both biomarkers were higher (0.916 vs 0.877 and 0.997 vs 0.985 respectively) than in younger patients (Table 3). When AUCs were compared in patients aged ≤55 years and > 55 years, the numerical values for AUCs were virtually the same.

A univariate logistic regression analysis showed that the odds ratio for the diagnosis of TPE significantly decreased with increasing age (OR = 0.62/10 years) and significantly increased with increasing level of all evaluated pleural fluid biomarkers (Table 4).

The results of bivariate regression analysis that included pleural fluid biomarker level and patients' age are presented in the last two columns of Table 4. In all biomarkers except ADA, age did not affect the OR for TP diagnosis (P value between 0.42 and 0.93). When pleural fluid ADA level was analysed together with patients' age, a relevant reduction in OR for TPE was found in older patients. Although the result was slightly higher than the level of statistical significance (P = 0.077, 95% CI 0.59–1.03), it should be stressed that for a given level of pleural fluid ADA, OR for TP may decrease as much as 22% for 10 years of age.

As age was found to be possibly relevant (on the verge of significance) for interpretation of pleural fluid ADA level, detailed results of the analysis of the predicted probability of TPE diagnosis depending on ADA level and patients' age are presented in Table 5. The highest probability of TPE, exceeding 93% was calculated for young patients (25-year-old) with pleural fluid ADA level between 80 and 100 U/L. The same pleural fluid ADA levels in 85-year-old patients were associated with approximately 17% lower probability of TPE. Importantly, the impact of age was even higher in lower pleural fluid ADA levels. In patients with ADA level 40 U/L the probability of TPE was 73.1% in the youngest age group, while in the oldest age group, it was almost 35% lower (Table 5).

Aggregated probability plots of TP depending on the pleural fluid level of ADA, IFN-γ, IP-10 and sFasL alone (without including patients' age) are shown in Fig. 1. For the most commonly used cut-off level of ADA 40U/L, a good discriminating TPE probability of 80% was found. IFN-γ plot (panel B) was characterized by the steepest increase in the probability of TPE at relatively low IFN-γ concentrations (0–300 pg/mL).

The graphs showing the prediction of TPE diagnosis depending on the patient's age are shown in Fig. 2. Curves representing older patients are shifting towards higher ADA values and lower prediction values.

4. Discussion

Our study showed that of the four selected sensitive and specific pleural fluid TP biomarkers, only the diagnostic yield of ADA might be affected by the patients' age. The predictive value of pleural fluid ADA level for TP decreased with patients' age and the highest probability of TP (> 90%) was found in patients younger than 40 years with ADA levels between 75 and 100 U/L. In contrast, the predictive values of pleural fluid IFN-γ, IP-10 and sFasL were not influenced by patients' age. As our study evaluated the relationship between patients' age and the diagnostic yield of not only ADA but also three other biomarkers of TPE, we believe it adds to the current knowledge on the diagnostic value of various biochemical tests in pleural fluid.

In terms of ADA, our result seems to be concordant with the results of several previous studies. Zaric et al. demonstrated a negative correlation between pleural fluid ADA activity and age (Pearson's r = −0.308, p = 0.001) in 121 patients with TPE and MPE [23]. As these authors did not perform a subanalysis in patients with TPE, we cannot compare our result found in this specific subgroup with the result of the above study. In this context, our results are similar to those presented by Abrao et al. These authors were not able to show any

Table 3
Diagnostic performance of different biomarkers in patients < 45 years old or ≥45 years old and ≤55 years old or > 55 years old. ADA – adenosine deaminase. IFN-γ - interferon gamma, IP-10 – interferon gamma-induced protein 10, sFasL – soluble Fas ligand. Cut-off values for ADA are expressed in U/L, while for the remaining biomarkers in pg/mL.

	Age < 45 years				Age ≥45 years				Age ≤55 years				Age > 55 years			
	AUC	Cut-off	Sens	Spec	AUC	Cut-off	Sens	Spec	AUC	Cut-off	Sens	Spec	AUC	Cut-off	Sens	Spec
ADA	0.877	35.9	91.3	83.3	0.916	23.3	88.9	88.2	0.901	35.9	93.1	88.5	0.900	23.3	86.2	86.9
IFN-γ	0.985	30.1	100	91,7	0.997	108	96.1	99.2	0.989	30.1	100	92.0	0.996	108	95	100
IP-10	0.966	20 362	94.1	91.7	0.950	4720	96.1	84.1	0.915	20 362	95.4	96.0	0.931	4720	95	83.8
sFasL	NA	NA	NA	NA	0.933	41.9	90.5	88.1	0.918	42.1	100	90.9	0.939	41.9	87.5	88.9

Table 4
The odds ratio of TPE diagnosis in relation to patients' age or the level of various biomarkers – univariate logistic regression analyses (column 2). The odds ratio of TPE diagnosis for each biomarker calculated on the basis of logistic regression model, when controlling for age (column 4).

Marker	OR (95% CI)	P	OR (95% CI) for age per 10 years adjusted for marker	P
Age (10 years)	0.62 (0.50–0.76)	< 0.0001	x	x
ADA (1 U/L)	1.08 (1.06–1.11)	< 0.0001	0.78 (0.59–1.03)	0.077
IFN-γ (1 pg/mL)	1.03 (1.02–1.05)	< 0.0001	1.10 (0.50–2.39)	0.815
IP-10 (1000 pg/mL)	1.24 (1.17–1.32)	< 0.0001	0.98 (0.70–1.39)	0.931
sFasL (1 pg/mL)	1.06 (1.03–1.09)	< 0.0001	0.87 (0.62–1.22)	0.415

significant correlation between pleural fluid ADA and age in 174 patients with TPE. In contrast, a significant negative correlation ($r = -0.445$) with age in the entire study group was found [22]. Contrary to our results and the above results by Abrao et al., a significant negative correlation between pleural ADA and age was demonstrated ($r = -0.254$, $P < 0.001$) in a large group of 182 patients with TP managed by Lee et al. [17]. However, these authors admitted that in as many as 40% of patients the diagnosis of TB pleurisy was only probable. This may significantly weaken the power of this publication. A significant correlation of age with pleural fluid ADA both in the whole study group and in a subgroup of patients with TP was reported by Tay et al. [21]. However, the correlation coefficient in the TPE group was significantly lower than in the entire group ($r = -0281$, $p = 0.011$ vs $r = -0.621$, $p < 0.001$).

When all patients in our study were classified according to age, the most relevant differences in the diagnostic performance of the four biomarkers were noted for ADA. Two different classifications were tested, using 45 and 55 years as the threshold levels. These values were applied to comply with the classifications used by other authors and to enable a direct comparison of the results. Similar to the study by Abrao et al., our study showed that the optimal diagnostic accuracy in patients < 45 years old can be achieved with higher cut-off ADA values than in older patients. The above values were 35.9 and 23.3 in our study, while 43 and 29 U/L in the study by Abrao et al. In both studies, younger groups were characterized by lower pleural fluid ADA AUC compared to older patients (0.877 vs. 0.916 in our study and 0.792 vs 0.91 in the Brazilian study [22]. Classification of patients as younger and older than 55 years revealed similar but not as significant differences as reported by Tay et al. The group below the age of 55 years had a similar AUC as the older group, while the best cut-off value was 35.9 for the younger and 23.3 U/L for the older group. The respective values in the study by Tay et al. were: AUC 0.887 and cut-off level 72 U/L for patients aged 55 years or younger, while AUC 0.959 and cut-off level 26 U/L for patients older than 55 years) [21,22].

A bivariate logistic regression analysis demonstrated that all biomarkers, as well as patients' age should be included in a statistical model estimating the probability of TPE. The odds ratio of TPE diagnosis in relation to patient's age and the level of various biomarkers varied from 0.62 to 1.26 with high level of significance. The analysis showed that when using ADA only, age should be considered as factor affecting prediction of TPE diagnosis. Although the P value did not reach statistical significance ($p = 0.077$), this value was distinctly different from the P levels for the remaining matched models of marker and age.

Our results were also expressed as the probability of TPE diagnosis in relation to pleural fluid ADA levels and patients' age. We believe, this method of data presentation might be complementary to ROC analysis and may help to understand the true role of age in the interpretation of

Table 5
Probability (%) of TPE in consecutive age clusters depending on the ADA adjusted for age value in entire group.

Age (years) \ ADA (U/L)	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	100	125	150
25	12.5	20.9	31.8	43.8	55.3	65.3	73.1	79.1	83.4	86.5	88.8	90.4	91.5	92.3	92.8	93.2	93.3	93.1	90.2	82.7
30	11.2	19.0	29.2	40.8	52.3	62.4	70.7	76.9	81.6	85.1	87.5	89.3	90.5	91.4	92.0	92.3	92.5	92.3	89.1	80.8
35	10.1	17.2	26.7	37.9	49.2	59.5	68.1	74.7	79.7	83.4	86.1	88.1	89.4	90.4	91.0	91.4	91.6	91.4	87.8	78.9
40	9.1	15.5	24.4	35.1	46.2	56.5	65.3	72.3	77.7	81.6	84.6	86.7	88.2	89.3	90.0	90.4	90.6	90.4	86.5	76.8
45	8.1	13.9	22.2	32.3	43.2	53.5	62.5	69.8	75.5	79.8	82.9	85.2	86.9	88.1	88.8	89.3	89.5	89.3	84.9	74.6
50	7.2	12.6	20.2	29.7	40.2	50.5	59.6	67.2	73.2	77.7	81.1	83.6	85.4	86.7	87.6	88.1	88.3	88.0	83.3	72.2
55	6.4	11.3	18.3	27.2	37.3	47.4	56.6	64.4	70.7	75.5	79.2	81.9	83.8	85.2	86.1	86.7	87.0	86.6	81.6	69.7
60	5.7	10.1	16.5	24.8	34.5	44.4	53.6	61.6	68.1	73.2	77.1	80.0	82.1	83.6	84.6	85.3	85.5	85.2	79.6	67.0
65	5.1	9.1	14.9	22.6	31.7	41.4	50.5	58.6	65.4	70.7	74.8	77.9	80.2	81.9	82.9	83.7	83.9	83.6	77.6	64.3
70	4.5	8.1	13.4	20.6	29.2	38.4	47.5	55.6	62.5	68.1	72.4	75.8	78.2	80.0	81.2	81.9	82.2	81.9	75.4	61.1
75	4.0	7.2	12.0	18.6	26.7	35.6	44.4	52.6	59.6	65.4	69.9	73.4	76.1	77.9	79.2	80.0	80.4	79.9	73.0	58.5
80	3.6	6.4	10.8	16.8	24.4	32.8	41.4	49.5	56.6	62.6	67.7	71.0	73.7	75.8	77.1	78.0	78.4	77.9	70.6	55.5
85	3.2	5.7	9.7	15.2	22.2	30.2	38.5	46.5	53.6	59.7	64.6	68.4	71.3	73.4	74.9	75.8	76.2	75.7	67.9	52.4

Bright and shaded fields show different probability of TPE in the evaluated population - □ < 49.9%, □ 50-79.9%, □ 80-89.9%, ■ 90-99.9%

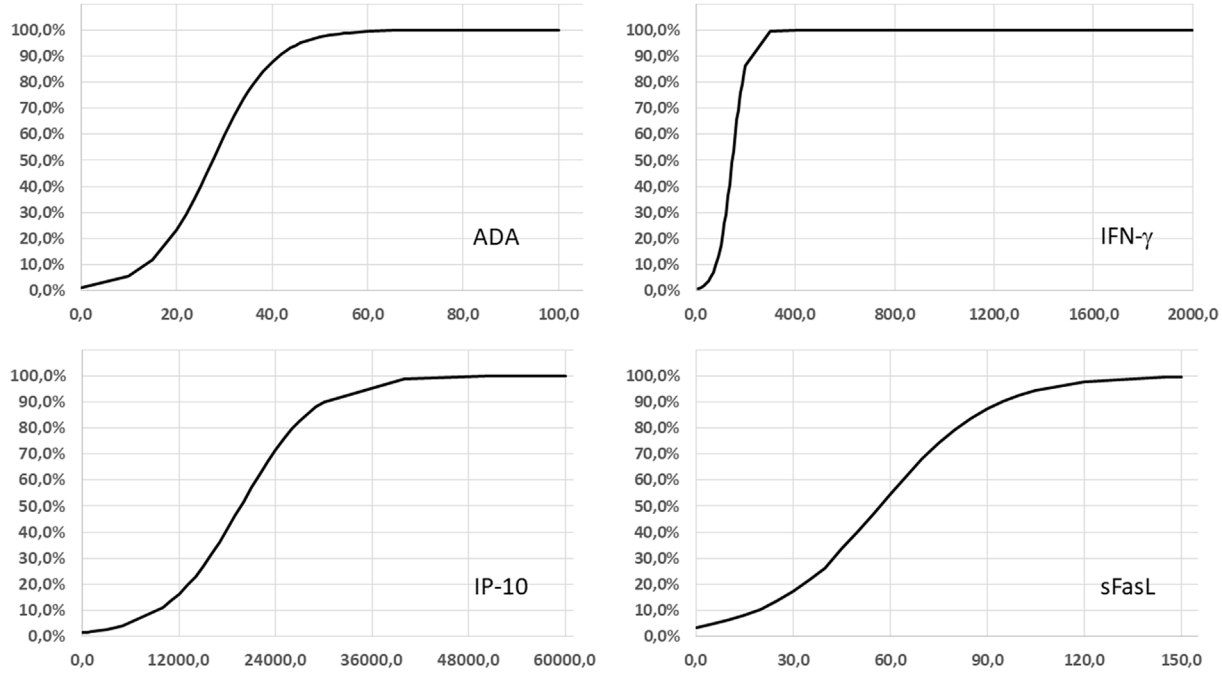

Fig. 1. Probability of the TPE diagnosis depending on the values of ADA, IFN-γ, IP-10, sFasL without considering the influence of age.

pleural fluid ADA level. Although AUC for ADA was higher in older patients (as discussed previously), the probability of TPE in patient with a given pleural fluid ADA level was lower in this group. This is mainly due to an increased probability of malignant pleural effusion in older patients. The highest probability of TPE, even exceeding 90%, was found in patients younger than 40 years with ADA levels between 65 and 125 U/L. It must be noted, however, that the above calculations are valid only for the populations with the prevalence of the underlying diseases similar to that in our study. This prevalence is not uncommon in pulmonary centres in low to median prevalence of TB setting. For example, in a French study by Michot et al., there were 32.7% of patients with TPE, 28.8% patients with MPE, 13.5% of patients with transudates and 12.5% of patients with PPE/PE. That group was recruited in 2001–2008 in France, in a hospital located in the southern district of Paris which in some aspects may correspond to the location of our centre in Warsaw, Poland [24].

The progressive decline in ADA activity with age seems to be an interesting issue. As the source of ADA are mainly macrophages and lymphocytes, age related decrease in pleural fluid ADA may reflect aging dysfunction of these cells as suggested by Hsu et al. [25]. Immune dysfunction with aging is believed to result from a functional decrease in the T-lymphocyte count (T-maturation disorder, aging of immunocytes or T-cell activation disorder) [14]. T-cell associated immunity deteriorates in elderly patients. The changes include a decline in the naïve T cells generation rate, shrinkage of the volume of the peripheral lymphoid tissue, decline of absolute and relative blood concentrations of naïve T cells, shortening of the average telomere length of T cells [14,26]. On the other hand, recent studies have demonstrated

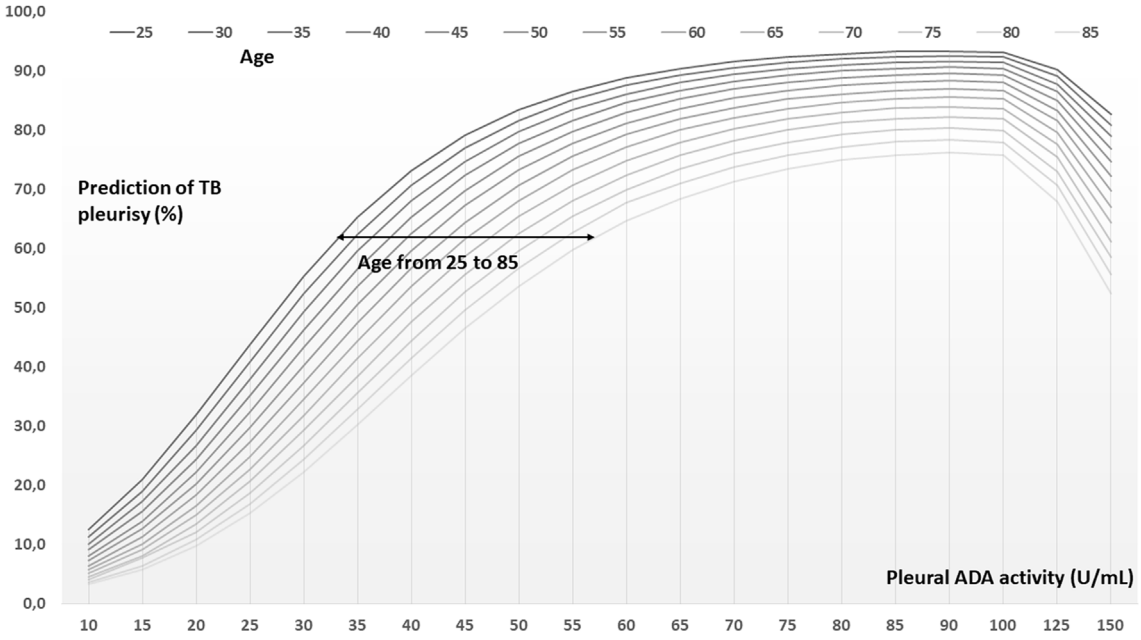

Fig. 2. Prediction values of ADA for the diagnosis of tuberculous pleurisy in individual age groups.

that pleural ADA activity is a sensitive marker of TPE also in HIV-positive patients, even in those with very low CD4-cell counts [27,28]. Thus, the precise mechanisms of the age associated decrease in pleural effusion ADA are still to be elucidated.

In contrast to ADA, we did not find any relevant relationships between pleural fluid levels of IFN-γ, IP-10 or sFasL and patients' age. Also, the diagnostic yield of the above biomarkers was not significantly affected by the age. As, to our knowledge, there were no earlier studies on the relationships between pleural fluid level or the diagnostic yield of IFN-γ, IP-10 or sFasL and patients' age, we could not compare our results with other reports. It should be emphasized, that all biomarkers selected for analyses together with ADA (IFN-γ, IP-10, and sFasL) are known to have a high diagnostic accuracy. This was demonstrated not only in original studies but also in their meta-analyses [29–34].

We are aware about some limitations of our study. First, this was a single centre study that included a moderate number of patients, thus reflecting a local epidemiological situation. On the other hand, the prevalence of pleural fluid causes in other European centres may be not significantly different (as discussed above). Second, although some statistical trends have been found, the number of patients may have been too low to obtain an adequate statistical power of calculations. This refers, in particular, to subgroup analyses. Therefore, it seems necessary to increase the number of patients in the subsequent studies what will enable an increase in the number of intra-group calculations. The reliability of calculations would certainly be higher, if a larger number of patients with different underlying diseases in small e.g. 5 or 10-year-old clusters had been available for analysis. It might be, however, argued that our study is a real life study and the enrolment of a larger number of patients with TPE (for example from special TB clinics) would be associated with a significant selection bias.

5. Conclusions

The study demonstrated that the diagnostic yield of pleural fluid ADA might be affected by patients' age. This phenomenon is associated with the need to apply a higher ADA cut-off values in younger patients. On the other hand, as the proportion of MPE increases with patients' age, the AUC for pleural fluid ADA as a biomarker of TP is higher in the older population. In a low/intermediate TB incidence setting, a young patient's age and a high pleural fluid ADA level are associated with very high probability of TP. This probability significantly decreases not only with decreasing pleural fluid ADA level but also with increasing patients age. In contrast, the predictive values of pleural fluid IFN-γ, IP-10 and sFasL are not affected by patients' age.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2018.11.004.

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