Interleukin 27 Could Be Useful in the Diagnosis of Tuberculous Pleural Effusions

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BACKGROUND: The diagnosis of tuberculous pleural effusion (TBPE) has some limitations. We studied the efficacy of interleukin-27 (IL-27) in the diagnosis of TBPE. METHODS: We measured IL-27, adenosine deaminase (ADA), ADA-2, interferon-gamma (IFNγ), and the ADA·IL-27 and ADA-2·IL-27 products in all the pleural effusion fluids. The diagnostic yield of IL-27 was evaluated with receiver operating characteristic curves. RESULTS: Of 431 pleural effusions, 70 were tuberculous, 146 were neoplastic, 58 were parapneumonic, 28 were empyemas, 88 were transudates, and 41 were other types. With a cutoff point of 0.55 ng/mL, IL-27 had a sensitivity of 91.4% and a specificity of 85.1%, which were significantly less than ADA, ADA-2, IFNγ, ADA·IL-27, or ADA-2·IL-27. The area under the receiver operating characteristic curve for IL-27 (0.963) was also significantly lower than that for the other markers, except for IFNγ. However, IL-27 improved the sensitivity of ADA and ADA-2 through ADA·IL-27 and ADA-2·IL-27 products (100% for both). CONCLUSIONS: IL-27 is less efficient than ADA and ADA-2 in the diagnosis of TBPE. However, ADA·IL-27 and ADA-2·IL-27 improve the diagnostic sensitivity of ADA and ADA-2, and thus could be useful in situations of high clinical suspicion and low ADA level. A value above the cutoff point of the latter is practically diagnostic of TBPE. *Key words: pleural disease; tuberculosis; interleukin.* [Respir Care 2014;59(3):399–405. © 2014 Daedalus Enterprises]

Introduction

Tuberculosis is one of the most frequent causes of pleural effusion in Spain.[1] Unfortunately, the conventional methods for diagnosing pleural inflammations have limitations, such as lack of yield by culture and staining, and wait-time to culture *Mycobacterium tuberculosis*.[2,3]

The analysis of pleural fluid interferon-gamma (IFNγ), adenosine deaminase (ADA), and its iso-enzymes, lysozyme, interleukin (IL), and lymphocyte subpopulations,[4-9] has, in some cases, improved the diagnostic yield of tuberculous pleural effusion (TBPE). This way, with the determination of ADA (a reference when evaluating the usefulness of new biomarkers in the diagnosis of TBPE), it has been questioned whether, in view of the elevated ADA in young people who live in areas with a high tuberculosis incidence, a pleural biopsy needs to be performed to establish the diagnosis.[10,11]

Several studies have shown that IL-27, along with IL-12, plays an important role in regulating human macrophage function during infection and thus impeding *M. tuberculosis* growth.[12,13] We found no studies of the usefulness of IL-27 in the diagnosis of TBPE, and therefore studied the efficacy of IL-27 in the diagnosis of TBPE,

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comparing it with ADA, ADA-2, IFNγ, and the products ADA·IL-27 and ADA-2·IL-27.

Methods

This prospective study was approved by the Clinical Research Ethics Committee of Galicia (study 2012/216). All subjects signed the informed consent prior to any study procedures. We screened all patients admitted to the Chest Diseases Department of Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain, from January 2008 to April 2012. Pleural effusions were diagnosed as tuberculous if: caseous necrotic granulomas were found in pleural tissue; Ziehl-Neelsen stain or Lowenstein culture of pleural effusion fluid or pleural tissue were positive; or Ziehl-Neelsen stain or Lowenstein culture of sputum samples were positive if the pleural effusion was accompanied by pulmonary infiltration. The diagnoses of other pleural effusions were made per the criteria of Villena-Garrido et al.[14]

Pleural fluid samples were taken via thoracocentesis, at admission, before any treatment, and underwent Ziehl-Neelsen staining, culture (in aerobic, anaerobic, and Lowenstein media), cytology, and biochemistry. Total cell counts were determined with a hematology system (Advia 2120, Siemens, Berlin, Germany). IL-27 was measured with an enzyme immunoassay (BioLegend, San Diego, California), per the manufacturer's protocol. ADA was measured with a coupled reaction with glutamate dehydrogenase and the rate of disappearance of nicotinamide adenine dinucleotide with high-energy hydrogen (NADH), measured at 340 nm in a spectrophotometer (Falcor 350, Menarini International, Florence, Italy).[15] ADA-2 was measured via inhibition with erythro-9-(2-hydroxy-3-nonyl) adenine.[16] IFNγ was measured with an enzyme-linked immunosorbent assay kit (IFNγ ELISA, IBL International, Hamburg, Germany, limit of detection 100 pg/mL, intra-assay reproducibility 4.5%, inter-assay reproducibility 5.7%). Aliquots of the pleural fluid were centrifuged immediately, for 15 min, at 1,500 g, at 4°C, and the supernatants were stored at −80°C. The closed pleural biopsies were performed with either a Cope or Abrams needle.[17,18]

Statistical Analysis

The Kolmogorov-Smirnov test was used to check the normality of data distribution, and non-normally distributed data were subjected to log transformations. The data are expressed as medians and 95% CIs. The groups were compared with the Bonferroni post hoc multiple comparison test. Diagnostic performance was analyzed with receiver operating characteristic curves.[19] The optimum cutoff point was considered the value that provided the greatest sum of sensitivity plus specificity. We calculated the optimum cutoff points, sensitivity, specificity, positive and negative likelihood ratios, and positive and negative predictive values with standard formulae. We used the method of DeLong et al to calculate the standard error of the area under the curve and the differences between areas under the curve.[20] For the calculation of the receiver operating characteristic curves and areas under the curve we used statistics software (MedCalc 12.2.1.0, MedCalc, Marakerke, Belgium).

QUICK LOOK

Current knowledge

Tuberculosis is a frequent cause of pleural effusion. The diagnosis of tuberculous pleural effusion is complicated by low yield and the prolonged culture time for *Mycobacterium tuberculosis*.

What this paper contributes to our knowledge

Interleukin-27 (IL-27) was significantly higher in tuberculous pleural effusion than in other pleural effusions, but IL-27 was less sensitive and specific for diagnosis of tuberculous pleural effusion than were adenosine deaminase (ADA) and ADA-2. However, the products ADA-2·IL-27 and ADA·IL-27 had better sensitivity than ADA or ADA-2, respectively, for diagnosing tuberculous pleural effusion.

Results

We screened 519 patients admitted to our hospital during the study period. We excluded 88 for not having a definitive diagnosis. The remaining 431 were classified in 6 diagnostic groups: TBPE, neoplastic pleural effusions, parapneumonic pleural effusions (PPE), empyema pleural effusions, transudate pleural effusions, and miscellaneous other pleural effusions (Fig. 1 and Table 1).

The distributions of the biomarkers in each group of pleural effusions are summarized in Table 2. For all the biomarkers the median of the tuberculous group differed significantly from those of the other groups in all cases. The diagnostic thresholds obtained via receiver operating characteristic curve analysis were: IL-27 0.55 ng/mL, ADA 56 U/L, ADA-2 44.5 U/L, IFNγ 108.2 pg/mL, ADA·IL-27 $7.6 \times 10^3 \cdot U \cdot ng/L^2$, and ADA-2·IL-27 $20.5 \times 10^3 \cdot U \cdot ng/L^2$. However, the receiver operating characteristic curves (Fig. 2) show that IL-27 separated TBPEs from the rest of the groups less well than did ADA, ADA-2, ADA·IL-27, or ADA-2·IL-27. The area under the IL-27 receiver operating characteristic curve (0.943) was significantly smaller than that of ADA (0.963), ADA-2 (0.991),

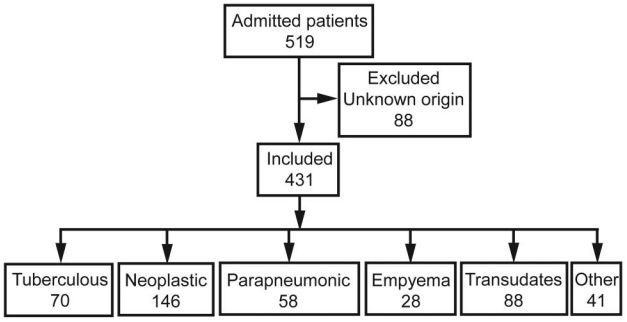

Fig. 1. Flow chart.

Table 1. Etiologies of Pleural Effusions in 431 Subjects in Spain

Etiology	no.
Tuberculous	70
Neoplastic	146
Bronchogenic carcinoma	72
Breast	20
Lymphoma	18
Stomach	5
Colon	5
Prostate	3
Melanoma	2
Thyroid	2
Uterus	2
Bladder	2
Pancreas	2
Phaeochromocytoma	2
Ovary	2
Esophagus	1
Thymus	1
Kidney	1
Mesothelioma	1
Uncertain	5
Parapneumonic	58
Empyema	28
Transudate	88
Heart failure	77
Hepatic hydrothorax	9
Hypoalbuminemia	2
Miscellaneous	41
Post-surgery	12
Hemothorax	9
Thoracic traumatism	8
Pulmonary thromboembolism	4
Hepatic abscess	3
Systemic lupus erythematosus	2
Subdiaphragmatic infection	1
Sudphrenic abscess	1
Chylothorax	1

ADA·IL-27 (0.991), or ADA-2·IL-27 (0.994), but not significantly different than IFNγ (0.953).

The IL-27, ADA, ADA-2 and IFNγ levels were significantly higher in the TBPE group than in the other pleural-effusion groups (Fig. 3). All the TBPEs had biomarker values above the cutoff points, except for 6 subjects for IL-27 (91.4%), 1 subject for ADA (98.6%), 3 subjects for ADA-2 (95.8%), and 7 subjects for IFNγ (90%). Sub-threshold levels of IL-27, ADA, ADA-2, and IFNγ were found in 85.1%, 93.6%, 97.5%, and 90.6%, respectively, in the non-tuberculous pleural effusions. The ADA·IL-27 and ADA-2·IL-27 values were also significantly higher in the TBPE group than in the rest of the groups. Both ADA·IL-27 and ADA-2·IL-27 correctly classified all the TBPEs, as well as 94.2% (ADA·IL-27) and 99.1% (ADA-2·IL-27) of the non-tuberculous pleural effusions.

Table 3 lists the numbers of misclassifications by each biomarker and group. From worst to best, IL-27 misclassified 13.9% of the effusions, IFNγ 9.5%, ADA and ADA·IL-27 5.8%, ADA-2 2.3%, and ADA-2·IL-27 0.9%. There were significant differences between ADA-2 and ADA-2·IL-27 and the rest, but not between themselves. The misclassification rate for IL-27 did not differ significantly from that of IFNγ, but was significantly greater than those of ADA, ADA-2, ADA·IL-27, and ADA-2·IL-27.

Table 4 lists the diagnostic performance of the biomarker: IL-27 had a sensitivity of 91.4%, a specificity of 85.1%, a positive likelihood ratio of 6.11, and an negative likelihood ratio of 0.10. The sensitivity of IL-27 was significantly lower than the other biomarkers, except for IFNγ (no significance). The sensitivity of both ADA·IL-27 and ADA-2·IL-27 was significantly higher than the rest, and there were no differences between them. The specificity of IL-27 was significantly lower than the other biomarkers, whereas the specificity of ADA-2·IL-27 and ADA-2 were significantly higher than that of the other biomarkers, and there were no differences between them. In the correlation study, statistical significance was observed only between IFNγ and ADA ($r = 0.435$, $P = .002$) and ADA-2 ($r = 0.322$, $P = 0.26$). IL-27 did not correlate with any of the biomarkers.

The pleural biopsy showed caseating granulomas in 76% of the TBPE (57/75) and the pleural fluid culture was positive in 25.3% of these. IFNγ was the only biomarker that had a significant association ($r = 0.48$, $P = .02$) with the pleural fluid culture (data not shown).

Discussion

IL-27 was significantly higher in TBPE than in the other pleural effusion groups, but IL-27 was less sensitive and specific than ADA or ADA-2. ADA-2·IL-27 and ADA·IL-27 improved the sensitivity over ADA and ADA-2, respectively, in the diagnosis of TBPE.

Table 2. Interleukin 27, Adenosine Deaminase, Interferon Gamma, and Adenosine Deaminase Products Levels in 431 Subjects With Pleural Effusion

	Type of Pleural Effusion						
	Tuberculous	Neoplastic	Parapneumonic	Empyema	Transudate	Miscellaneous	Unknown
Subjects, n	70	146	58	28	88	41	88
Male, %	47.1	59.6	67.2	67.9	60.2	51.2	61.5
Age, mean ± SD y	39.5 ± 22.1	67.3 ± 13.4*	68.8 ± 18.3*	62.4 ± 16.1*	73.3 ± 13.8*	63.7 ± 13.3*	73.4 ± 12.8
IL-27, ng/mL	1.18 (0.99–1.34)	0.29* (0.26–0.32)	0.31* (0.26–0.35)	0.12† (0.01–0.18)	0.33* (0.26–0.39)	0.33* (0.26–0.37)	
ADA, U/L	117.5 (98.5–125.5)	23.3* (821.5–25.4)	25* (22.0–27.0)	45.5* (27.7–157.1)	12* (10.–13.1)	23* (17.9–28.9)	24 (22–26)
ADA-2, U/L	106.4 (92.6–116.5)	19.2* (16.7–21.3)	19* (15.5–24.3)	19.5* (15.1–21.5)	11* (9.0–12.8)	17.4* (12.4–27.8)	25.1 (18–30.5)
IFNγ, pg/mL	1,021 (611.8–2400)	15.2* (8.8–28.7)	30.6* (17.6–64.5)	15.1* (6.6–27.6)	22.2* (15.0–28.6)	17.9* (7.9–28.2)	22.9 (11.6–110.7)
ADA·IL-27, 10^3·U·ng/L^2	117.6 (106.5–135.8)	5.9* (5.0–7.0)	7.1* (5.7–9.1)	5* (2.1–8.0)	3.3* (2.9–4.2)	6.3* (4.7–9.4)	
ADA-2·IL-27, 10^3·U·ng/L^2	94.6 (87.6–125.6)	4.4* (3.6–5.5)	5.5* (3.9–5.8)	0.9* (0.2–3.1)	2.9* (2.4–3.8)	5.5* (3.1–9.1)	

Values are median and 95% CI unless otherwise indicated. The values in the tuberculous group were significantly different from the values in all 5 other groups.
* $P < .001$.
† $P = .001$.
IL = interleukin
ADA = adenosine deaminase
IFNγ = interferon gamma

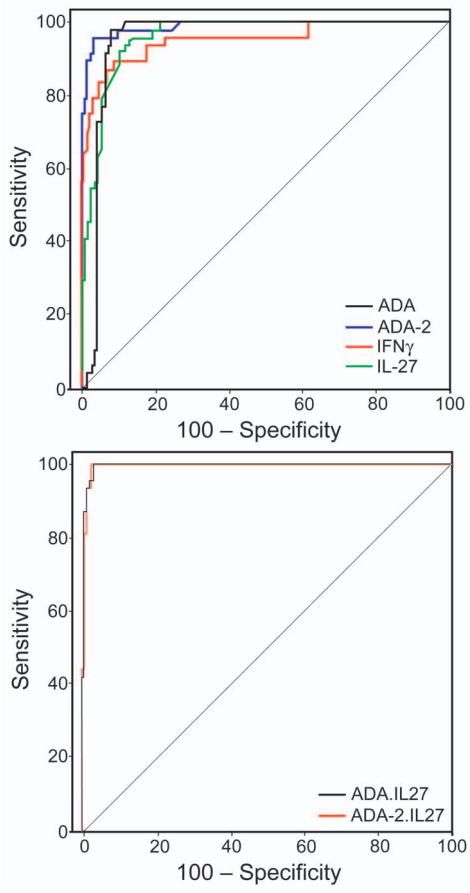

Fig. 2. Receiver operating characteristic curves for diagnosis of tuberculous pleural effusions with: (top) interleukin-27 (IL-27), adenosine deaminase (ADA), adenosine deaminase 2 (ADA-2), and interferon gamma (IFNγ); (bottom) ADA·IL products.

Macrophages represent a bridge between the innate and adaptive immunity. In the early innate response, macrophages recognize microbes by their pattern recognition receptors such as toll-like receptors, leading to the transcription of IL-27 and other cytokines. Once secreted, the cytokines stimulate the adaptive immune response in lymphocytes.[21] IL-27, a heterodimeric cytokine consisting of the Epstein-Barr-virus-induced gene 3 products and the p28 polypeptide, is produced earlier, by active antigen presenting cells in response to the microbial infection. It is capable of inducing CD4+ clonal proliferation in naive CD4+ T cells, but not in memory cells. T cells synergize with IL-12 in the production of IFNγ by naive CD4 T cells.[22]

Our results indicate that the majority of TBPEs can be identified by high IL-27 (sensitivity 91.4%), although its specificity is somewhat lower (85.1%). That sensitivity and specificity are significantly lower than that of the other biomarkers, except for the sensitivity of IFNγ. All the non-tuberculosis pleural effusions had values higher than the established cutoff point (21 neoplastic, 10 parapneumonic, 3 empyema, 13 transudate, and 7 miscellaneous), always higher in number than any of the other biomarkers, except ADA in the empyema group.

Fourteen of the 21 misclassified neoplastic pleural effusions were lymphomas, which accounted for 77.8% of them (14/18) (median 1.05, 95% CI 0.7–1.26). No significant differences were found in this subgroup as regards TBPE ($P = .26$). This could explain the observation that splenocytes of mice with different types of cancer produce high concentrations of IFNγ in blood, which could pro-

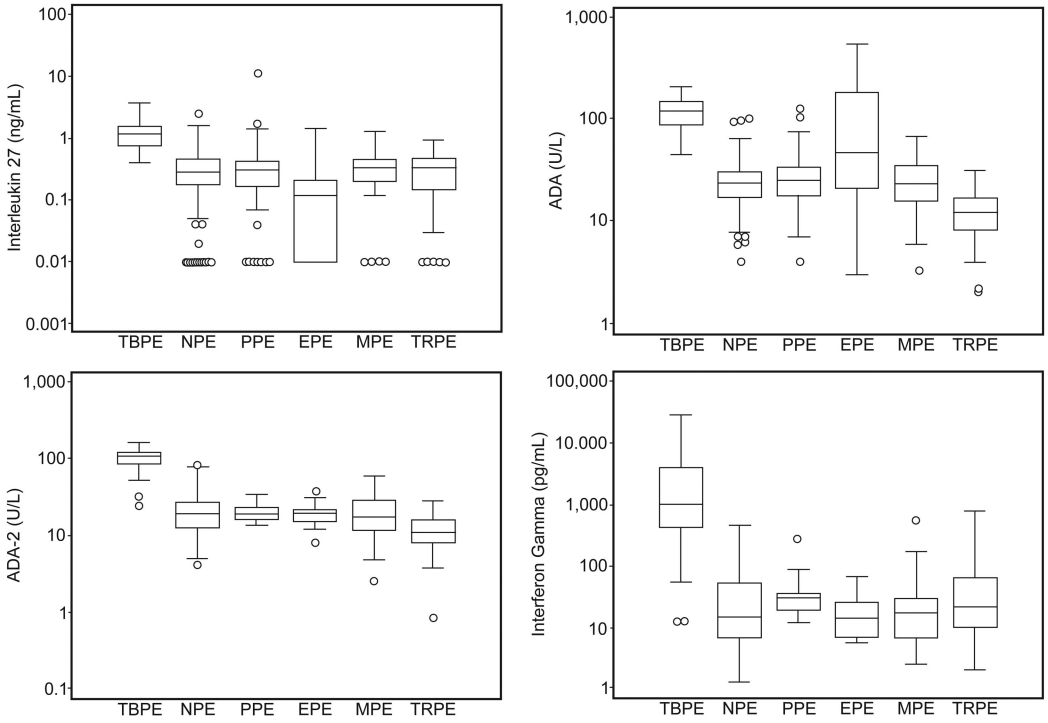

Fig. 3. Pleural fluid concentrations of interleukin 27, adenosine deaminase (ADA), adenosine deaminase-2 (ADA-2), and interferon gamma in 6 etiologies of pleural effusion: tuberculous (TBPE), neoplastic (NPE), parapneumonic (PPE), empyema (EPE), miscellaneous (MPE), and transudates (TRPE). Pleural ADA, ADA-2, IFNγ, and IL-27 are plotted on a log scale. In each data bar, the middle line represents the median, the bottom and top of the bar represent the 25th and 75th percentiles, the whiskers represent the minimum and maximum values excluding outliers, and the circles represent outliers.

Table 3. Numbers of Misclassified Pleural Effusions

	Biomarker and Cutoff Value					
	ADA > 56 U/L	ADA-2 > 44.5 U/L	IFNγ > 108.2 pg/mL	IL-27 > 0.55 ng/mL	ADA·IL-27 >27.6 10^3·U·ng/L^2	ADA-2·IL-27 > 20.5 10^3·U·ng/L^2
Tuberculous	1 (1.4)	3 (4.2)	7 (10)	6 (8.6)	0 (0)	0 (0)
Neoplastic	5 (3.4)	7 (4.8)	20 (13.7)	21 (14.4)	14 (9.6)	3 (4.9)
Parapneumonic	5 (8.6)	0 (0)	6 (10.3)	10 (17.2)	6 (10.3)	0 (0)
Empyema	13 (46.4)	0 (0)	0 (0)	3 (10.7)	2 (7.1)	0 (0)
Miscellaneous	1 (2.4)	2 (4.9)	4 (9.8)	7 (10.2)	3 (7.3)	1 (4.2)
Transudate	0 (0)	0 (0)	4 (4.5)	13 (14.8)	0 (0)	0 (0)
Total	25 (5.8%)	12 (2.3%)	41 (9.5%)	60 (13.9)	25 (5.8)	4 (0.9)
P for comparisons with total misclassified with ADA-2	.02		< .001	< .001	.02	.17
P for comparison with total misclassified with IL-27	.001	< .001	.057		.001	< .001

Values are number and percent.
ADA = adenosine deaminase
IFNγ = interferon gamma
IL = interleukin

duce high levels of IL-27.[23,24] In infectious diseases, IL-27 shows anti-inflammatory activity by suppressing the response of the T-helper-2 cells,[25] which could explain the high IL-27 values in some parapneumonic and empyema pleural effusions. In any case, no previous studies have measured IL-27 in pleural fluid, so we need to study our

Table 4. Diagnosic Performance of Interleukin 27, Adenosine Deaminase, Interferon Gamma, and Adenosine Deaminase/Interleukin 27 Products for Tuberculous Pleural Effusion

	Threshold	Sensitivity %	Specificity %	Positive Likelihood Ratio	Negative Likelihood Ratio	Positive Predictive Value	Negative Predictive Value
IL-27	> 0.55 ng/mL	91.4	85.1	6.11	0.10	54.2	98.1
ADA	> 56 U/L	98.6	93.4	14.9	0.01	74.2	99.7
ADA-2	> 44.5 U/L	95.7	97.5	38.3	0.04	88.2	99.2
IFNγ	> 108.2 pg/mL	90	90.9	9.90	0.11	64.9	97.9
ADA·IL-27	> 27.6 10^3·U·ng/L^2	100	93.4	15.04	0.00	73.7	100
ADA-2·IL-27	> 20.5 10^3·U·ng/L^2	100	98.9	90.25	0.00	94.6	100

ADA = adenosine deaminase
IL = interleukin
IFNγ = interferon gamma

hypothesis on why IL can be elevated in non-tuberculous pleural effusions.

The yield of the rest of the biomarkers was similar to previous studies.[8,26-28] In the present study the overall misclassification rate of ADA-2 was significantly lower than that of ADA, although this could be explained by the increased number of empyema pleural effusions in our series.

We previously found that the increase in ADA and its ADA-2 isoenzyme in TBPE was a reflection of macrophage activation due to greater activation of CD4+ lymphocytes in pleural fluid.[6] Several meta-analyses and subsequent studies[29-32] have indicated that IFNγ has a high yield in the diagnosis of TBPE (sensitivity 89%, specificity 97%). However, in the present study, as in others by our group,[4,8] its yield has been lower, although within the range obtained in the meta-analyses (sensitivity 64–100%, specificity 86–100%). This could be due to disparities between the studies included in the meta-analyses, as regards the subjects (range 21–595), the observed prevalence of tuberculosis (13.8–74.2%), or the methods of determination.

We have found that ADA·IL-27 and ADA-2·IL-27 improve the diagnostic yield over ADA and its isoenzyme ADA-2, respectively, in TBPE. The rationale for using ADA·IL-27 and ADA-2·IL-27, being both elevated in pleural effusion fluid, was that they could better differentiate TBPE from those who only have one of the biomarkers above the cutoff. Thus, we expected that the products of TBPE were higher than those of non-tuberculous pleural effusions. The yield of these products should be higher than that obtained from the requirement of values of ADA (or ADA-2) and IL-27 above the cutoff point, because this would lose sensitivity, although it would gain specificity. By contrast, requiring the achievement of only one of 2 values will increase sensitivity at the expense of decreasing specificity. There were significant differences in the sensitivity for both products (ADA 98.6% vs ADA·IL-27 100%, $P = .004$, ADA-2 95.7% vs ADA-2·IL-27 100%, $P = .001$), but not in the specificity (both ADA and ADA·IL-27 93.4%, ADA-2 97.5% vs ADA-2·IL-27 98.9%). Only 4 subjects (1.1%) with a non-tuberculous pleural effusions had elevated ADA-2·IL-27, with all the TBPEs well classified, so a value > 20.5 10^3·U·ng/L^2 of this biomarker in pleural fluid is practically diagnostic of TBPE (positive likelihood ratio 90.9).

As expected, there were significant relationships between ADA, ADA-2, and IFNγ. There was no significant relationship between IL-27 and IFNγ ($r = -0.24$). Human macrophages infected by *M. tuberculosis*, in response to IL-12, secrete IFNγ. Robinson et al observed increased IFNγ production after neutralizing IL-27 with a soluble receptor (sIL-27R).[13] This suggests that the activities of IL-27 and IL-12 could be antagonistic to the macrophages. However, both synergize in the production of IFNγ in CD4+ and natural killer T cells.[13] IL-27, unlike IL-12, induces T-helper-1 cell differentiation, even in the presence of anti-IFNγ neutralizing antibodies, which suggests that IFNγ is not necessary for this. On the other hand, once IL-12 has been produced in sufficient quantities (it is produced after IL-27), it appears that this may not be necessary for the maintenance of the T-helper-1 cells.[33,34] This could explain the lack of correlation that we found between IL-27 and IFNγ. In turn, the fact that increased IL-27 may not be necessary once the immune response is initiated could explain why its diagnostic yield may be less than the other biomarkers we studied. Further study is required to confirm this hypothesis associated with the IL-12, IL-27, and IFNγ levels in pleural fluid.

Conclusions

The observed elevation of IL-27 in TBPE suggests that IL-27 plays a role in the immune response to *M. tuberculosis* infection, although IL-27 is less efficient in the diagnosis of TBPE than is ADA or ADA-2. However, the

products ADA·IL-27 and ADA-2·IL-27 improve the sensitivity (but not the specificity) of ADA and ADA-2, respectively, so a value of the latter above the cutoff point is practically diagnostic of TBPE. Thus, ADA·IL-27 and ADA-2·IL-27 should be of use in situations of high clinical suspicion and low ADA. Further studies are needed to elucidate the role of IL-27 in this process.

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