RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

TITLE: Interleukin 27 could be useful in the diagnosis of tuberculous pleural effusions

RUNNING HEAD: Interleukin 27 and pleural effusions

Luis Valdés, Esther San José, Lucía Ferreiro, Antonio Golpe, Francisco Gude, José M. Álvarez-Dobaño, Marco F. Pereyra, María E Toubes, Francisco J. González-Barcala.

Valdés L.
Servicio de Neumología
Complejo Hospitalario Clínico-Universitario de Santiago
Grupo Interdisciplinar de Investigación en Neumología, Instituto de Investigaciones Sanitarias de Santiago (IDIS)
Santiago de Compostela, Spain
luis.valdes.cuadrado@sergas.es

San José E.
Servicio de Análisis Clínicos
Complejo Hospitalario Clínico-Universitario de Santiago
Grupo Interdisciplinar de Investigación en Neumología, Instituto de Investigaciones Sanitarias de Santiago (IDIS)
Santiago de Compostela, Spain
sanjosec@yahoo.com

Ferreiro L.
Servicio de Neumología
Complejo Hospitalario Clínico-Universitario de Santiago
Santiago de Compostela, Spain
lu_micino@hotmail.com

Golpe A. Servicio de Neumología
Complejo Hospitalario Clínico-Universitario de Santiago
Grupo Interdisciplinar de Investigación en Neumología, Instituto de Investigaciones Sanitarias de Santiago (IDIS)
Santiago de Compostela, Spain
antonio.golpe.gomez@sergas.es

Gude F.
Unidad de Epidemiologia Clínica.
Complejo Hospitalario Clínico-Universitario de Santiago
Grupo Epidemiologia de Enfermedades Frecuentes, Instituto de Investigaciones Sanitarias de Santiago (IDIS)
Santiago de Compostela, Spain
francisco.gude.sampedro@sergas.es

Álvarez-Dobaño JM. Servicio de Neumología
Complejo Hospitalario Clínico-Universitario de Santiago
Grupo Interdisciplinar de Investigación en Neumología, Instituto de Investigaciones Sanitarias de Santiago (IDIS)

Santiago de Compostela, Spain
jad01po@hotmail.com

Pereyra MF. Servicio de Neumología
Complejo Hospitalario Clínico-Universitario de Santiago
Santiago de Compostela, Spain
marcofrancisco.pereyra.barrionuevo@sergas.es

Toubes ME. Servicio de Neumología
Complejo Hospitalario Clínico-Universitario de Santiago
Santiago de Compostela, Spain
maria.elena.toubes.navarro@sergas.es

González-Barcala FJ. Servicio de Neumología
Complejo Hospitalario Clínico-Universitario de Santiago
Grupo Interdisciplinar de Investigación en Neumología, Instituto de Investigaciones Sanitarias de Santiago (IDIS), Spain
francisco.javier.gonzalez.barcala@sergas.es

Corresponding Author:
Valdés L.
Servicio de Neumología
Complejo Hospitalario Clínico-Universitario de Santiago
Travesia da Choupana s/n
15706 Santiago de Compostela, Spain
luis.valdes.cuadrado@sergas.es

All authors have signed a conflict of interest form. There are no conflicts of interest.

Funding information: Our work was carried-out without external funding.

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Specific contributions of each contributor to the paper:

Luis Valdés: Literature search, Study design, Analysis of data, Manuscript preparation, Review of manuscript.

Esther San-José: Literature search, Study design, Analysis of data, Manuscript preparation, Review of manuscript.

Lucía Ferreiro: Literature search, Review of manuscript.

Antonio Golpe: Data collection, Review of manuscript.

Francisco Gude: Study design, Analysis of data, Review of manuscript

José M. Álvarez-Dobaño: Data collection, Review of manuscript.

Marco F. Pereyra: Data collection, Review of manuscript.

María E Toubes: Data collection, Review of manuscript.

Francisco J. González-Barcala: Literature search, Study design, Analysis of data, Manuscript preparation, Review of manuscript.

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

ABBREVIATION LIST

ADA: adenosine deaminase

AUC: area under the curve

EBI3: Epstein-Barr virus-induced gene 3

EPE: empyema pleural effusion

GLDH: glutamate dehydrogenase

IFNγ: interferon-gamma

IL: interleukins

IL-27: Interleukin-27

MPE: miscellaneous pleural effusion

NLR: negative likelihood ratio

NPE: neoplastic pleural effusion

PE: pleural effusion

PLR: positive likelihood ratio

PPE: parapneumonic pleural effusion

ROC: receiver operating characteristics

TB: Tuberculosis

TBPE: tuberculous pleural effusions

TRPE: transudate pleural effusion

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Abstract Word count: 210.

Manuscript Word count: 2,502.

Introduction: The diagnosis of tuberculous pleural effusions (TBPE) has some limitations.

Objective: To evaluate the efficacy of Interleukin-27 (IL-27) in the diagnosis of TBPE.

Methods: A total of 431 pleural effusions were classified as tuberculous (n=70), neoplastic (n=146), parapneumonic (n=58), empyemas (n=28), miscellaneous (n=41), and transudates (n=88). IL-27, adenosine deaminase (ADA), ADA-2, interferon-gamma (IFNγ) and the ADA·IL-27 and ADA-2·IL-27 products were measured in all of the fluids. The diagnostic yield of IL-27 was evaluated using operating characteristics (ROC) analysis.

Results: With a cut-off 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 the rest of the parameters. The area under the ROC curve (0.963) was also significantly less than the rest of the markers, except for IFNγ. However, IL-27 improves the sensitivity of the 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, using the products of ADA·IL-27 and ADA-2·IL-27, helps to improve the diagnostic sensitivity of ADA and ADA-2, thus it could be useful in situations of high clinical suspicion and low levels of ADA. A value above the cut-off point of the latter is practically diagnostic of TBPE.

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

KEY WORDS: Pleural disease; Tuberculosis; Interleukin.

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Introduction

Tuberculosis (TB) is one of the most frequent origins of pleural effusion (PE) in our country.[1] Unfortunately, the conventional methods for the diagnosis of these pleural inflammations have certain limitations, such as lack of yield by culture and staining, or the waiting time to obtain growth of the *Mycobacterium Tuberculosis*.[2,3]

The analysis of certain biochemical parameters in the pleural fluid (interferon-gamma (IFNγ), adenosine deaminase (ADA) and its iso-enzymes, lysozyme, interleukins (IL), and lymphocyte sub-populations),[4-9] has helped, in some cases, to improve the diagnostic yield of tuberculous pleural effusion (TBPE). This way, with the determination of ADA (a reference when evaluating the usefulness of new biochemical parameters in the diagnosis of TBPE), it has been questioned whether, in view of the elevated values of this parameter found 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 *Mycobacterium Tuberculosis* growth.[12,13] As far as we know, there are no studies that have evaluated its usefulness in the diagnosis of TBPE.

The aim of this study is to evaluate the efficacy of IL-27 in the diagnosis of TBPE, comparing it with that of ADA, ADA-2 and IFNγ, and to determine whether on its own, or using the products ADA·IL-27 and ADA-2·IL-27, it can help improve the diagnostic yield of TBPEs.

Material and Methods

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

A prospective study was conducted on all patients admitted to the Chest Diseases Department of the Complejo Hospitalario Universitario de Santiago (Santiago de Compostela, Spain), from January 2008 to April 2012. Pleural effusions were diagnosed as tuberculous if (1) caseous necrotic granulomas were found in pleural biopsy tissue samples, (2) Ziehl-Neelsen stains or Lowenstein cultures of effusion or biopsy tissue samples were positive, or (3) Ziehl-Neelsen stains or Lowenstein cultures of sputum samples were positive if the pleural effusion was accompanied by pulmonary infiltration. Diagnoses of other pleural effusions were made according to previously defined criteria.[14]

Pleural fluid samples were taken by thoracocentesis at admission before starting any treatment. The pleural fluid samples were sent to microbiology (for Ziehl-Neelsen staining and cultures in aerobic, anaerobic and Lowenstein media), cytology and biochemistry. Total cell counts were determined with a Siemens ADVIA 2120 Haematology System (Siemens Healthcare Diagnostics Inc., Deerfield, USA). The IL-27 concentration (ng/mL) was measured using an enzyme immunoassay (BioLegend Inc., San Diego, CA, USA) using the manufacturer's protocol. ADA, expressed in U/L, was determined using a coupled reaction with glutamate dehydrogenase (GLDH) and the rate of the disappearance of NADH measured at 340 nm in a FALCOR 350 (Menarini International S.r.l., Florence),[15] and the ADA-2 isoenzyme was determined by inhibition with erythro-9-(2-hydroxy-3-nonyl)adenine,[16] IFNγ was determined (pg/mL) using an ELISA kit (Interferon-gamma ELISA- IBL International GmbH, Hamburg Germany) (limit of detection: 100 pg/ml, intra-assay reproducibility 4.5%, inter-assay 5.7%). Aliquots of the pleural fluid specimens were centrifuged immediately for 15 minutes at 1.500 x g at 4 ^{o}C, and the supernatants were stored at -80 ^{o}C. The closed pleural biopsies were performed either with Cope or Abrams needles.[17,18]

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

All patients signed the informed consent before any procedure was performed (thorax CT with contrast, thoracocentesis, pleural biopsy, thoracoscopy or thoracotomy). The protocol was evaluated and approved by the Clinical Research Ethics Committee of Galicia (registry 2012/216).

Statistical analysis

Kolmogorov-Smirnov tests were used to check distributional normality; non-normal distributions were subjected to log transformations. The data were expressed as median (95% confidence interval). Groups were compared using a post hoc multiple comparison test (Bonferroni). For the evaluation of the diagnostic performance of parameters studied as markers for the differential diagnosis between TBPEs and non-TBPEs, receiver operating characteristics (ROC) analysis was performed for all significant differences between groups.[19] Receiver operator characteristics curves were generated by plotting the sensitivity against 1 – specificity, and the area under the curve (AUC) with 95% confidence intervals (95%CIs) was calculated. The optimum cut-off point from the ROC analysis was established by selecting the value that provided the greatest sum of sensitivity and specificity. For the optimum cut-off point provided by each ROC analysis, sensitivity, specificity positive and negative likelihood ratios (PLR and NLR, respectively), and positive and negative predictive value, were calculated using standard formulae. We used the method of DeLong et alfor the calculation of the Standard error of the Area under the curve (AUC) and of the difference between two AUCs.[20] For the calculation of the ROC curves and AUCs, we used the MedCalc version 12.2.1.0 software (MedCalc, Marakerke, Belgium).

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Results

A total of 519 patients admitted to our hospital during the study period were analysed, with 88 being excluded due to not having a definitive diagnosis. The remaining 431 were classified in six diagnostic groups: TBPE, neoplastic pleural effusions (NPE), parapneumonic pleural effusions (PPE), empyema pleural effusions (EPE), miscellaneous pleural effusions (MPE), and transudate pleural effusions (TRPE) (Figure 1). The aetiology of the pleural effusions is shown in Table 1.

The distributions of the biochemical parameters in each group of pleural effusions are summarised in Table 2. For all these parameters, the median of the tuberculous group differed significantly from those of the other groups in all cases. The diagnostic thresholds obtained by the ROC analysis for IL-27, ADA, ADA-2, IFNγ and the products of ADA·IL-27 and ADA-2·IL-27 were 0.55 ng/mL, 56 U/L, 44.5 U/L, 108.2 pg/mL, 7.6 $(10^3 \cdot U \cdot ng)/L^2$ and 20.5 $(10^3 \cdot U \cdot ng)/L^2$ respectively. However, the ROCs of the diagnostic parameters (Figure 2) showed that IL-27 separated TBPEs from the rest of the groups less well than ADA, ADA-2, ADA·IL-27 and ADA-2·IL-27. The area under the IL-27 ROC, 0.943, was significantly smaller than the area of these parameters (0.963, 0.991, 0.991 and 0.994 respectively). On the other hand, there were no significant differences as regards that of IFNγ (0.953).

The IL-27, ADA, ADA-2 and IFNγ levels were significantly higher in the TBPE group than in the rest (Figure 3). All the TBPEs had values above the established cut-off points, except for 6 in the case of IL-27 (91.4%), 1 for the ADA (98.6%), 3 for ADA-2 (95.8%), and 7 in the case of 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-TBPE. The values of the products of ADA·IL-27 and ADA-2·IL-27 were also

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

significantly higher in the TBPE group than in the rest of the groups. Both products correctly classified all the TBPEs, as well as 94.2% (ADA.IL-27) and 99.1% (ADA-2.IL-27) of the non-TBPEs.

Table 3 lists the numbers of misclassifications by each parameter and group. From worst to best, IL-27 misclassified 13.9% of effusions, IFNγ 9.5%, ADA and ADA·IL-27 product 5.8%, ADA-2 2.3% and ADA-2·IL-27 product 0.9%. Significant differences were found between these last two parameters and the rest, but not between themselves. The misclassification rate of IL-27 did not differ significantly from that of IFNγ, but was significantly greater than those of ADA, ADA-2 and ADA·IL-27 and ADA-2·IL-27 products.

Table 4 lists other performance parameters, emphasising that IL-27 had a sensitivity of 91.4%, a specificity of 85.1%, a PLR of 6.11 and an NLR of 0.099. Between the studied parameters, the sensitivity of IL-27 was significantly lower than the rest, except for IFNγ (no significance). The sensitivity of both products was significantly higher than the rest and with no differences between them. The specificity of IL-27 was significantly lower than the rest of the parameters, whereas that of the ADA-2·IL-27 product and ADA-2 were significantly higher than that of the other parameters, with no differences between them. In the correlation study, a statistical significance was only observed between ADA and IFNγ (r =-0.0018, $P<.0001$). IL-27 did not correlate with any of the parameters analysed.

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. The IFNγ was the only biomarker that had a significant association (r =0.477, $P<.0214$) with the pleural fluid culture (data not shown).

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Discussion

These results confirm that IL-27 levels are significantly higher in TBPE than in the rest of the groups studied, but are less efficient than the levels of the established markers ADA and ADA-2. On the other hand, IL-27, using the ADA- 2·IL-27 and ADA·IL-27 products helps to improve the sensitivity of ADA and ADA-2, respectively, in the diagnosis of TBPE.

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 (TLRs), 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 (EBI3) 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 DC4+ T-cells but not in memory cells. T-cells synergise with IL-12 in the production of IFNγ by naive CD4 T-cells.[22]

Our results confirm that the majority of the TBPEs could be identified by their high levels of IL-27 (sensitivity, 91.4%), although its specificity is somewhat lower (85.1%). These results were significantly lower than the rest of the parameters studied (except the sensitivity for IFNγ). The effusions from all non-TB groups had values higher than the established cut-off point (21 NPE, 10 PPE, 3 EPE, 7 MPE and 13 TRPE), always higher in number than any of the other parameters, except ADA in the EPEs. It is worth pointing out that 14 of the 21 misclassified NPEs 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 sub-group as regards TBPE (*P*=.260). This could explain why it has

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

been observed that the splenocytes of mice with different types of cancer produce high concentrations of IFNγ in blood, and this, in turn, could produce high levels of IL-27.[23,24] In the case of infectious diseases, it has been observed that IL-27 shows anti-inflammatory activity by suppressing the response of the Th2 cells,[25] which could explain why high values are found in some PPE and EPE. In any case, as there are no previous studies in which IL-27 has been determined in pleural fluid, it is not possible to compare our results with those of other authors, or to verify our hypothesis on why this IL can be elevated in non-TBPEs.

The yield of the rest of the parameters was similar to previous studies.[8,26-28] On this occasion, the overall misclassification rate of ADA-2 is significantly lower than that of ADA, although this could be explained by the increased number of EPE in our series.

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

We have used the products of ADA·IL-27 and ADA-2·IL-27 to verify if IL-27 can contribute to improve the diagnostic yield of ADA and its isoenzyme ADA-2, respectively, in TBPEs. The rationale for using these products, being elevated both

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

parameters in these pleuritis, was because they could better differentiate TBPE from those who only have one of the parameters above the cut-off. Thus, we would expect that the products of TBPE were higher than those of non-TBPE. The yield of these products should be higher than the one obtained from the requirement of values of ADA (or ADA-2) and IL-27 above the cut-off point because this would lose sensitivity, although would gain specificity. By contrast, requiring the achievement of only one of two 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=.00397-; ADA-2 95.7% vs ADA-2·IL-27 100% - P=.0001-) 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 patients (1.1%) with a non-TBPE had elevated values of ADA-2·IL-27 with all the TBPEs well classified, as such that a value >20.5 $(10^3 \cdot U \cdot ng)/L^2$ of this parameter in pleural fluid is practically diagnostic of TBPE (PLR of 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 =-0.237). The human macrophages infected by *Mycobacterium Tuberculosis*, in response to IL-12, secrete IFNγ. Robinson et al observed an increase in the production of IFNγ, after neutralising IL-27 by means of a soluble receptor (sIL-27R).[33] This suggests that, the activities of IL-27 and IL-12 could be antagonistic in the macrophages. However, both synergise in the production of IFNγ in CD4+ and NK T-cells.[33] IL-27, unlike IL-12, induces Th1 differentiation, even in the presence of anti-IFNγ neutralising antibodies, which suggests that IFNγ is not necessary for this. On the other hand, once the 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 Th1.[34,35] This latter could explain the lack a correlation that we found between IL-27 and IFNγ. In turn, the fact that increased levels

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

of IL-27 may not be necessary once the immune response is initiated, could explain why its diagnostic yield may be less than the rest of the parameters used. Further studies will be required to confirm this hypothesis associated with the IL-12, IL-27 and IFNγ levels in pleural fluid.

In conclusion, the observed elevation of IL-27 in TBPEs is consistent with this interleukin in playing a role in the immune response to infection by *Mycobacterium tuberculosis* although it is less efficient in the diagnosis of TBPEs than ADA and ADA-2. However, IL-27 helps to improve the sensitivity (but not the specificity) of ADA and ADA-2 by using the ADA·IL-27 and ADA-2·IL-27 products, respectively, as such that a value of the latter above the cut-off point is practically diagnostic of TBPE, thus these products could be of use in situations of high clinical suspicion and low levels of ADA. Further studies would be needed to try to elucidate the real role that IL-27 plays in this process.

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

References

1. Valdes L, Alvarez D, Valle JM, Pose A, San Jose E. The etiology of pleural effusions in an area with high incidence of tuberculosis. Chest 1996;109(1):158-162.
2. Valdes L, Alvarez D, San Jose E, Penela P, Valle JM, García-Pazos JM et al. Tuberculous pleurisy: a study of 254 patients. Arch Intern Med 1998;158(18):2017-2021.
3. Escudero Bueno C, Garcia Clemente M, Cuesta Castro B, Molinos Martín L, Rodríguez Ramos S, González Panizo A, Martínez Glez-Río J.. Cytologic and bacteriologic analysis of fluid and pleural biopsy specimens with Cope's needle. Study of 414 patients. Arch Intern Med 1990;150(6):1190-1194.
4. Valdes L, San Jose E, Alvarez D, Sarandeses A, Pose A, Chomón B, et al. Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme, and interferon gamma. Chest 1993;103(2):458-465.
5. Valdes L, Pose A, San Jose E, Martinez Vazquez JM. Tuberculous pleural effusions. Eur J Intern Med 2003;14(2):77-88.
6. Valdes L, San Jose E, Alvarez D, Valle JM. Adenosine deaminase (ADA) isoenzyme analysis in pleural effusions: diagnostic role, and relevance to the origin of increased ADA in tuberculous pleurisy. Eur Respir J 1996;9(4):747-751.
7. Fontan-Bueso J, Verea-Hernando H, Garcia-Buela JP, Dominguez-Juncal L, Martin-Egana MT, Montero-Martinez MC. Diagnostic value of simultaneous determination of pleural adenosine deaminase and pleural lysozyme/serum lysozyme ratio in pleural effusions. Chest 1988;93(2):303-307.

8. Valdes L, San Jose E, Alvarez Dobano JM, Golpe A, Valle JM, Penela P, González Barcala FJ. Diagnostic value of interleukin-12 p40 in tuberculous pleural effusions. Eur Respir J 2009;33(4):816-820.
9. San Jose ME, Valdes L, Saavedra MJ, De Vega JM, Alvarez D, Viñuela J, et al. Lymphocyte populations in tuberculous pleural effusions. Ann Clin Biochem 1999;36(Pt 4):492-500.
10. Valdes L, Alvarez D, San Jose E, Juanatey JR, Pose A, Valle JM, et al. Value of adenosine deaminase in the diagnosis of tuberculous pleural effusions in young patients in a region of high prevalence of tuberculosis. Thorax 1995;50(6):600-603.
11. Valdes L, San Jose ME, Pose A, Gude F, González-Barcala FJ, Alvarez-Dobaño JM, Sahn SA. Diagnosing tuberculous pleural effusion using clinical data and pleural fluid analysis A study of patients less than 40 years-old in an area with a high incidence of tuberculosis. Respir Med 2010;104(8):1211-1217.
12. Robinson CM, Nau GJ. Interleukin-12 and interleukin-27 regulate macrophage control of Mycobacterium tuberculosis. J Infect Dis 2008;198(3):359-366.
13. Robinson CM, O'Dee D, Hamilton T, Nau GL. Cytokines involved in interferon-γ production by human macrophages. J Innate Immun 2010;2(1):56-65.
14. Villena-Garrido V, Ferrer Sancho J, Hernandez-Blasco L, de Pablo Gafas A, Pérez Rodríguez E, Rodríguez Panadero F, et al. Diagnosis and treatment of pleural effusion. Arch Bronconeumol 2006;42(7):349-72.
15. Ellis G, Goldberg DM. A reduced nicotinamide adenine dinucleotide-linked kinetic assay for adenosine deaminase activity. J Lab Clin Med 1970;76(3):507-517.
16. Giusti G, Gakis C. Temperature conversion factors, activation energy, relative substrate specificity and optimum pH of adenosine deaminase form human serum and tissues. Enzyme 1971;12(4):417-425.

17. Cope C. New pleural biopsy needle. JAMA 1958;167(9):1107-1108.
18. Abrams LD. New inventions: a pleural biopsy punch. Lancet 1958;271(7010):30-31.
19. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39(4):561-577.
20. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44(3):837-845.
21. Pirhonen J, Sirén J, Julkunen I, Matikainen S. IFNγ regulates Toll-like receptor-mediated IL-27 gene expression in human macrophages. J Leukoc Biol 2007;82(5):1185-1192.
22. Pflanz S, Timans JC, Cheung J, Rosales R, Kanzler H, Gilbert J, et al . IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells. Immunity 2002;16(6):779-790.
23. Oniki S, Nagai H, Horikawa T, Furukawa J, Belladonna ML, Yoshimoto T, et al. Interelukin-23 and interleukin-27 exert quite different antitumour and vaccine effects on poorly immunogenic melanoma. Cancer Res 2006;66(12):6395-6404.
24. Hisada M, Kamiya S, Fujita K, Belladonna ML, Aoki T, Koyanagi Y et al. Potent antitumour activity of interleukin-27. Cancer Res 2004;64(3):1152-1156.
25. Villarino AV, Huang E, Hunter CA. Understanding the pro- and anti-inflammatory properties of IL-27. J Immunol 2004;173(2):715-720.
26. Gopi A, Madhavan SM, Sharma SK, Sahn SA. Diagnosis and treatment of tuberculous pleural effusion in 2006. Chest 2007;131(3):880-889.

27. Porcel JM, Esquerda A, Bielsa S. Diagnostic performance of adenosine deaminase activity in pleural fluid: a single-center experience with over 2,100 consecutive patients. Eur J Intern Med 2010;21(5):419-423.

28. Liang QL, Shi HZ, Wang K, Qin SM, Qin XJ. Diagnostic accuracy of adenosine deaminase in tuberculous pleurisy: a meta-analysis. Respir Med 2008;102(5):744-754.

29. Jiang J, Shi HZ, Liang QL, Qin SM, Qin XJ. Diagnostic value of interferon-γ in tuberculous pleurisy. Chest 2007;131(4):1133-1141.

30. Greco S, Girardi E, Masdangelo R, Capoccetta GB, Saltini C. Adenosine deaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis. Int J Tuberc Lung Dis 2003;7(8):777-786.

31. Dheda K, Van Zyl-Smit RN, Sechi LA, Badri M, Meldau R, Meldau S, et al. Utility of quntitative T-cell responses versus unstimulated interferón-γ for the diagnosis of pleural tuberculosis. Eur Respir J 2009;34(5):1118-1126.

32. Dheda K, Van-Zyl Smit RN, Sechi LA, Badri M, Meldau R, Symons G, et al. Clinical diagnostic utility of IP-10 and LAM antigen levels for the diagnostic of tuberculous pleural effusions in a high burden setting. PloS One 2009;4(3):e4689.

33. Robinson CM, O´Dee D, Hamilton T, Nau GJ. Cytokines involved in interferon-γ production by human macrophages. J Innate Immun 2000;2(1):56-65.

34. Hunter CA, Kastelein R. Interleukin-27: balancing protective and pathological immunity. Immunity 2012;37(6):960-969.

35. Owaki T, Asakawa M, Morishima N, Hata K, Fukai F, Matsui M, et al. A role for IL-27 in early regulation of Th1 differentiation. J Immunol 2005;175(4):2191-2200.

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Figure Legends

Figure 1. Flow chart of the studied patients

Figure 2. Receiver operating characteristics of the parameters (A) and products (B) studied, for diagnosis of tuberculous pleural effusions

Figure 3. Box plots of pleural fluid concentrations of A) interleukin 27 (IL-27); B) adenosine deaminase (ADA); C) adenosine deaminase-2 (ADA-2) and D) interferon gamma (IFNγ) in different aetiologies of pleural effusion Pleural ADA, ADA-2, IFNγ and IL-27 levels are plotted on a log scale. The central box represents the values from the lower to the upper quartile (25th-75th percentiles). The middle line represents the median. A line extends from the minimum to the maximum value. Outside values are displayed as separate points

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Table 1. Aetiology of pleural effusions

Etiology	n
Tuberculous	70
Neoplastic	146
Bronchogenic carcinoma	72
Breast	20
Lymphoma	18
Stomach	5
Colon	5
Prostate gland	3
Melanoma	2
Thyroid	2
Uterus	2
Bladder	2
Pancreas	2
Phaeochromocytoma	2
Ovary	2
Oesophagus	1
Thymus	1
Kidney	1
Mesothelioma	1
Uncertain	5
Parapneumonic	58
Empyemas	28
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
Chylotorax	1
Transudates	88
Heart failure	77
Hepatic hydrotorax	9
Hypoalbuminaemia	2

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Table 2. Descriptive statistics of diagnostics parameters considered for each type of pleural effusion

	Tuberculous	Neoplastic	Parapneumonic	Empyema	Miscellaneous	Transudates	Unknown
Subjects, n	70	146	58	28	41	88	88
Males, %	47.1	59.6	67.2	67.9	51.2	60.2	61.5
Age, yrs	39.5±22.1	67.3±13.4*	68.8±18.3*	62.4±16.1*	63.7±13.3*	73.3±13.8*	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* (0.26-0.37)	0.33* (0.26-0.39)	
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)	23* (17.9-28.9)	12* (10.-13.1)	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)	17,4* (12.4-27.8)	11* (9.0-12.8)	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)	17.9* (7.9-28.2)	22.2* (15.0-28.6)	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)	6.3* (4.7-9.4)	3.3* (2.9-4.2)	
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)	5.5* (3.1-9.1)	2.9* (2.4-3.8)	

Data are presented as mean±SD or median (95% confidence interval). ADA: adenosine deaminase; IFN: interferon; IL: interleukin; ADA·IL-27: ADA·IL-27 product; ADA-2·IL-27: ADA-2·IL-27 product. For all parameters, values in the tuberculous group differ significantly from values in the other five groups (*: P <.001; #: P=.001)

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Table 3. Numbers of misclassified effusions of each group, for each diagnostic parameter studied

	ADA	ADA-2	IFNγ	IL-27	ADA·IL-27	ADA-2·IL-27
Cut-off	>56 U/L	>44.5 U/L	>108.2 pg/mL	>0.55 ng/mL	>27.6 $(10^3 \cdot U \cdot ng)/L^2$	>20.5 $(10^3 \cdot U \cdot ng)/L^2$
TBPE	1 (1.4)	3 (4.2)	7 (10)	6 (8.6)	0 (0)	0 (0)
NPE	5 (3.4)	7 (4.8)	20 (13.7)	21 (14.4)	14 (9.6)	3 (4.9)
PPE	5 (8.6)	0 (0)	6 (10.3)	10 (17.2)	6 (10.3)	0 (0)
EPE	13 (46.4)	0 (0)	0 (0)	3 (10.7)	2 (7.1)	0 (0)
MPE	1 (2.4)	2 (4.9)	4 (9.8)	7 (10.2)	3 (7.3)	1 (4.2)
TRPE	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-value[#]	0.0149		<0.0001	<0.0001	0.0149	0.1718
p-value[¶]	0.0001	<0.0001	0.0570		0.0001	<0.0001

Data are presented as n or n (%). ADA: adenosine deaminase; IL: interleukin; IFN: interferon; ADA·IL-27: ADA·IL-27 product ; ADA-2·IL-27: ADA-2·IL-27 product ; TBPE: tuberculous pleural effusion; NPE: neoplastic pleural effusion; PPE: parapneumonic pleural effusion; EPE: empyema pleural effusion; MPE: miscellaneous pleural effusion; TRPE: transudate pleural effusion; NS: non-significant; [#]: for comparisons with total misclassified with ADA-2; [¶]: for comparison with total misclassified with IL-27

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

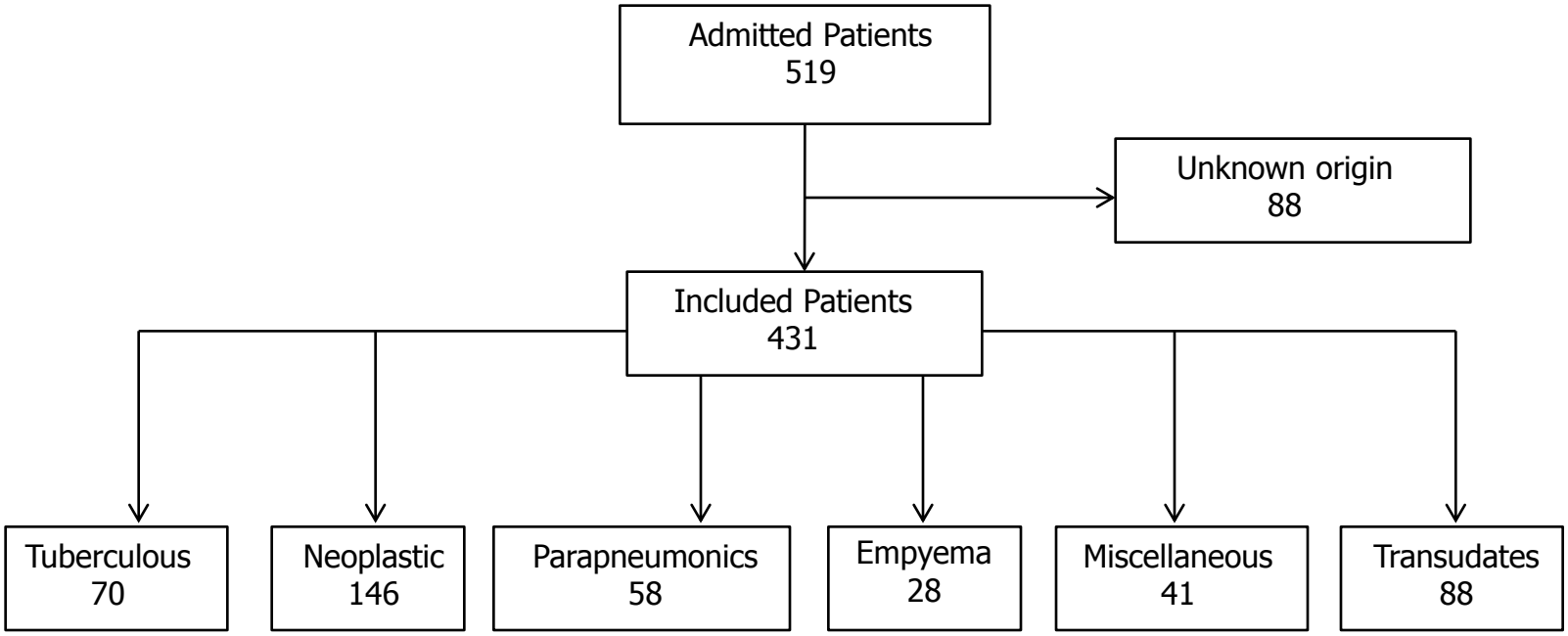
Table 4. Performance measures for diagnosis of tuberculous pleural effusions by each diagnostic parameter with the stated thresholds

	Threshold	Sensitivity, %	Specificity, %	PLR	NLR	PPV	NPV
IL-27, ng/mL	>0.55	91.4	85.0	6.11	0.10	54.2	98.1
ADA, U/L	>56	98.6	93.4	14.9	0.01	74.2	99.7
ADA-2, U/L	>44.5	95.7	97.5	38.3	0.04	88.2	99.2
IFNγ, pg/mL	>108.2	90	90.9	9.90	0.11	64.9	97.9
ADA·IL-27, $(10^3 \cdot U \cdot ng)/L^2$	>27.6	100	93.4	15.04	0.00	73.7	100
ADA-2·IL-27, $(10^3 \cdot U \cdot ng)/L^2$	>20.5	100	98.9	90.25	0.00	94.6	100

PLR: positive likelihood ratio; NLR: negative likelihood ratio; ADA: adenosine deaminase; IL: interleukin; IFNγ: interferon gamma. PPV: positive predictive value. NPV: negative predictive value.

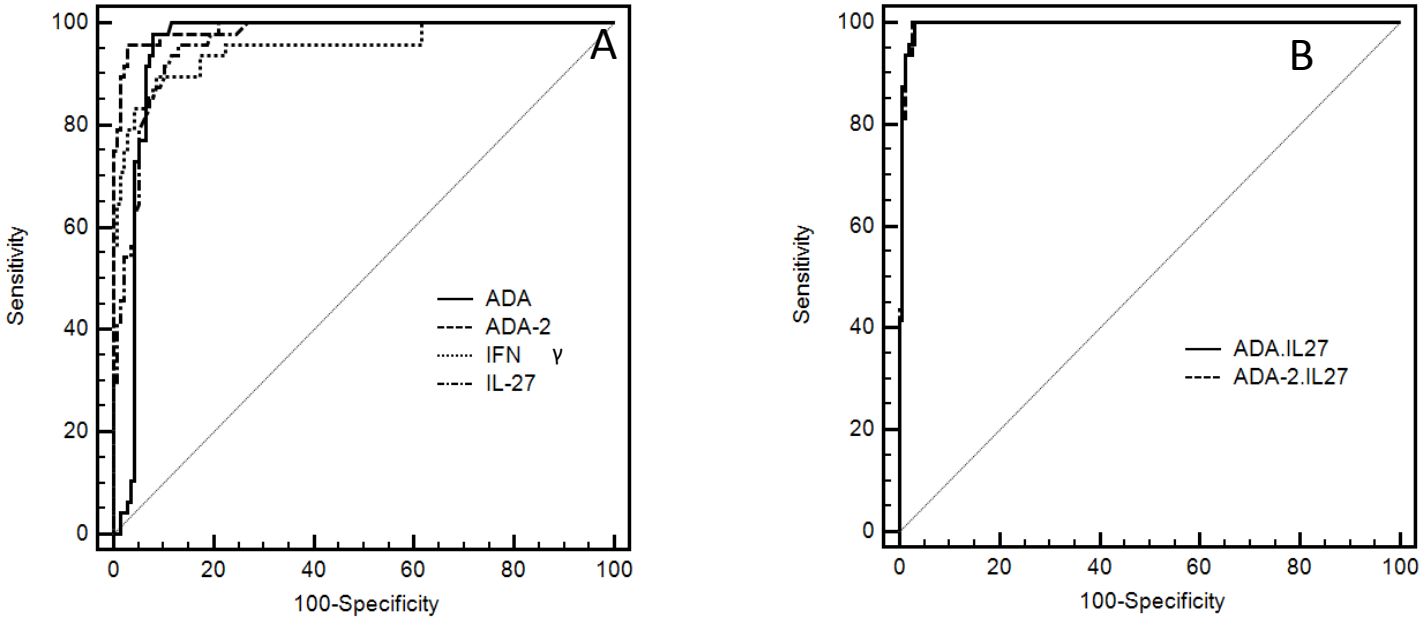
RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Figure 1



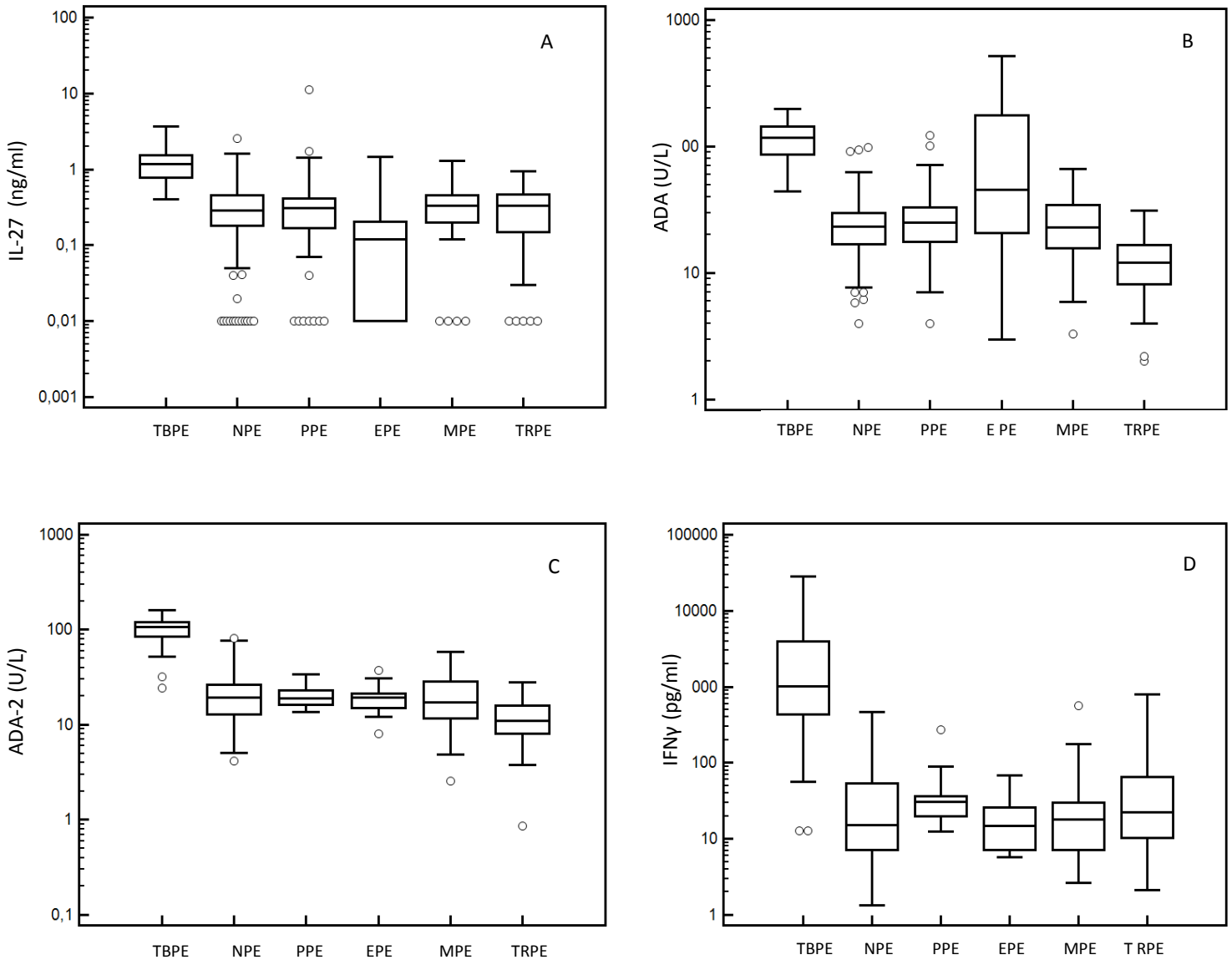
RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Figure 2



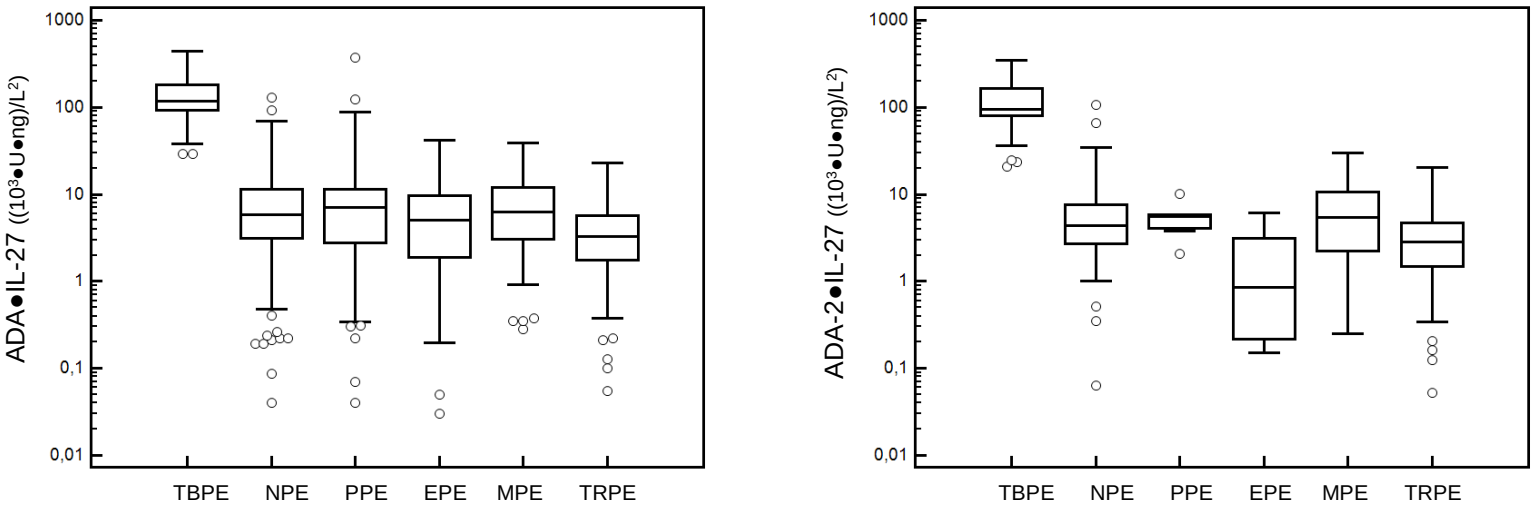
RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Figure 3

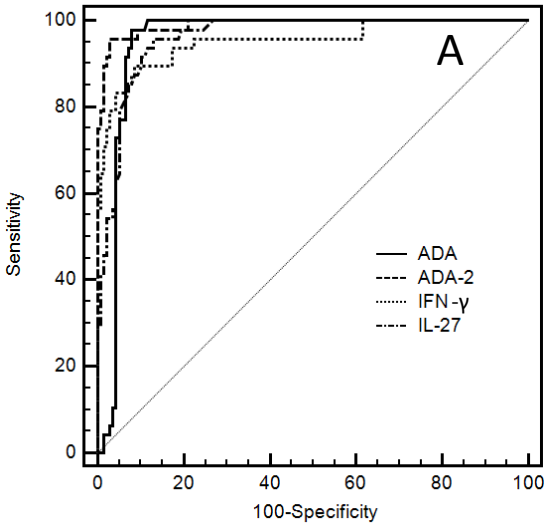


RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

Figure 4



RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749



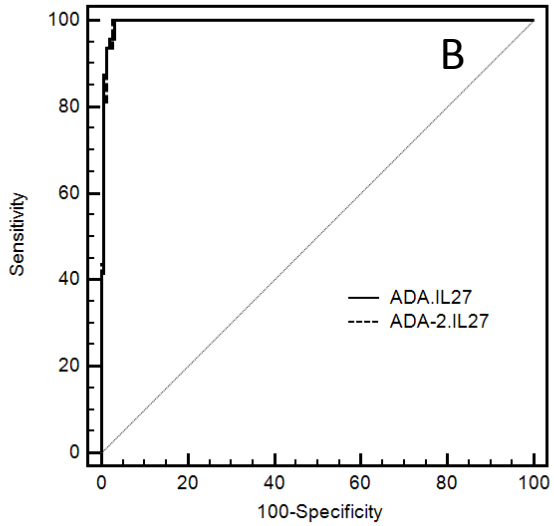


Fig. 2

RESPIRATORY CARE Paper in Press. Published on August 20, 2013 as DOI: 10.4187/respcare.02749

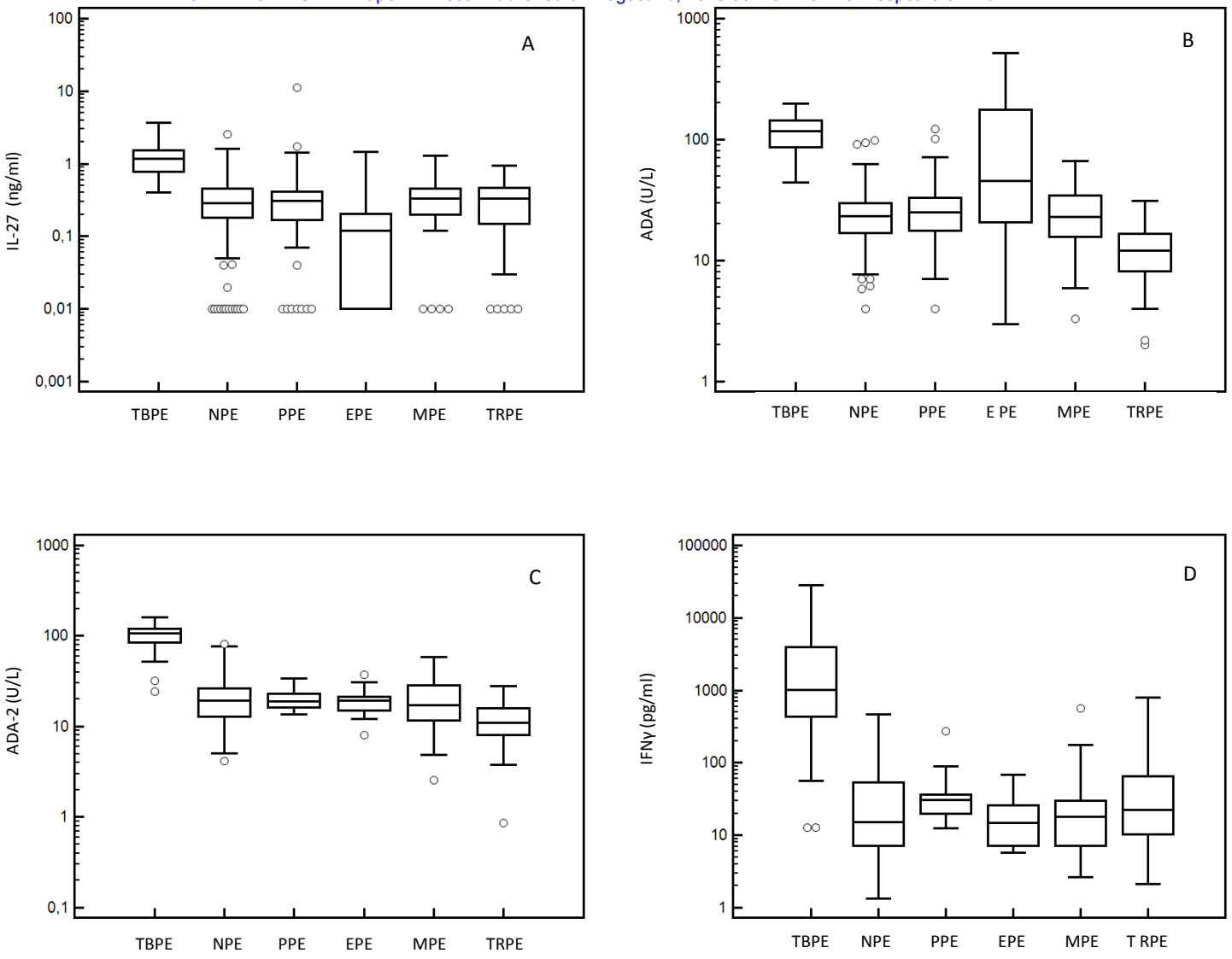


Fig. 3