

# Cytokine response as a biomarker for early diagnosis and outcome prediction of stem cell transplant recipients and acute leukemia patients with invasive aspergillosis

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## Abstract

We aimed to determine the role of serum cytokine expression in invasive aspergillosis (IA) diagnosis and outcome prediction in hematologic patients. In this multicenter study, serum cytokines (IL6, IL10, INF-gamma, IL12, IL4, TNF-alpha, IL17, and IL23) were prospectively recruited from all consecutive patients with hematologic malignancies at IA diagnosis and compared to control patients matched by center, age, baseline disease, and therapeutic regimen. We included 36 patients with IA and 36 controls. Serum levels of IL6 and IL10 cytokines on day 0 were significantly increased in patients with IA when compared to controls ( $P = 0.001$  and  $P = 0.025$ , respectively), even in those who were neutropenic. No differences were observed for the other cytokines. IL6 and IL10 predicted IA with an area under the ROC curve of 0.74 (95% CI 0.62–0.86) and 0.64 (95% CI 0.51–0.77), respectively. The best cutoff point in predicting IA was 20.85 pg/ml for IL6 (sensitivity 72.2%; specificity 77.8%; PPV 76.5% and NPV 73.7%), and 0.045 pg/ml for IL10 (sensitivity 62.9%; specificity 63.9%; PPV 62.9% and NPV 63.9%). IL6 levels were associated with increased mortality, with the best cutoff value being 65.59 pg/ml in mortality prediction. In conclusion, in addition to current tests in place, IL6 and IL10 levels—as measured in plasma—may help clinicians diagnose IA. High levels of IL6 at IA diagnosis are related with worse outcomes.

## Lay summary

We evaluated the role of serum cytokine expression in invasive aspergillosis (IA) diagnosis and outcome. Serum levels of IL6 and IL10 are increased in patients with IA compared to controls, and IL6 levels are associated with mortality.

**Keywords:** Invasive aspergillosis, immunological biomarkers, cytokines, outcomes

## Introduction

Invasive aspergillosis (IA) is a life-threatening disease that affects severely immune compromised patients.<sup>1</sup> Early initiation of active systemic antifungal therapy is key in improving outcomes.<sup>2,3</sup> However, early IA diagnosis remains a challenge for physicians. Despite the availability of high-resolution thoracic computerized tomography (CT) and fungal biomarkers, clinical presentation can be insidious; the clinical and radiological spectrum are widely diverse; and diagnostic tests with sufficient sensitivity and specificity are lacking. Additionally, assessing the clinical response of a fungal infection remains challenging, in patients with several other clinical conditions.

In this setting, using immunological markers for early diagnosis and clinical follow-up is appealing as a concept. The development of IA is a multiphase pathway that involves a

host immune response comprising several steps against infection.<sup>4,5</sup> In the early stages of aspergillosis, the host immune response is mediated by innate cells that amplify their response via cytokines secretion. It is attractive to speculate that some cytokines stimulated by the innate immune system may rapidly increase, marking the host response against infection. We, therefore, hypothesize that such cytokines could prove the index of early IA diagnosis, alongside the support of fungal biomarkers and/or CT scan results. Additionally, we hypothesize that those patients with initially higher or longer cytokine expression will suffer worse clinical outcomes.

We sought to describe the relationship between serum cytokine expression and IA diagnosis and prognosis. We also evaluated factors related with worse outcomes in these patients.

## Methods

### Setting, patients, and study design

We performed a prospective, multicenter matched case-control study of all hospitalized patients with acute leukemia (AL) and/or allogeneic hematopoietic stem cell transplantation (HSCT) recipients from four tertiary teaching institutions in Spain. The study period took place between January 2011 and December 2014. All consecutive patients admitted to the hematologic departments were evaluated and followed-up by one or more of the study investigators, recognized as experts in managing these types of patients. Cases and controls were then identified. Cases were defined as those patients presenting proven or probable IA in accordance with definitions set forth by the European Organization for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycosis Study Group (EORTC/MSG).<sup>6</sup> When a patient with AL and/or allogeneic HSCT presented signs or symptoms clinically suggestive of IA, standard diagnostic methods (fungal culture, serum galactomannan, high-resolution CT scan, and/or bronchoscopy) were performed following the attending physician decision. Serum was also obtained and stored at that timepoint to detect cytokines. Only samples from those patients finally defined as cases underwent analysis. The control population consisted of consecutive individuals admitted to the same center as the case population, with the following characteristics: similar age ( $\pm 5$  years), identical baseline disease, and identical therapeutic regimen. Controls were included in the study on the same day when cases were diagnosed with IA. The reference day for controls was considered as that when the patient started treatment for the underlying disease.

The following variables were prospectively recorded in a computerized database: demographic characteristics, underlying disease, clinical features, diagnostic test results, cytokine determination, therapy, and outcomes. This study was approved by the three institutional review boards.

### Cytokine measurements

For all patients with IA, blood samples were collected to determine cytokine levels on day 0, day 2+, day 7+, day 14+, and day 21+ since IA diagnosis. In controls, only cytokines at day 0 were obtained. To determine circulating levels of cytokines, the blood samples collected into EDTA tubes were immediately centrifuged and the plasma fraction was then stored at a temperature of  $-80^{\circ}\text{C}$ . Assays were performed by one of the study authors who was blind to clinical details of individual patients. Circulating concentrations of IL4, IL6, IL10, IL12, TNF, and IFN- $\gamma$  were measured with flow cytometry employing cytometric bead array technology. These cytokines were selected due to technical feasibility and their previously documented relationship with the response to fungal infection.<sup>4,7</sup> Samples were analyzed using a BD FACSCalibur flow cytometer (BD Biosciences) per the manufacturer's instructions. The detection limit was 1.4, 1.6, 1.13, 0.6, 1.2, and 0.8 pg/ml, respectively. IL23 concentration was measured with the commercial ELISA assay Quantikine® ELISA Human IL-23 Immunoassay (R&D Systems Inc., Minneapolis, MN, USA) (detection limit: 6.8 pg/ml). IL17 concentration was measured with commercial IL-17A Human ELISA Kit (Invitrogen) (detection limit: 0.5 pg/ml) per the manufacturer's instructions. IL6 levels above 43.5 pg/ml were defined as elevated.<sup>8</sup>

### Statistical analysis

A sample size calculation was done to evaluate the number of patients needed to detect differences in cytokine patterns at admission between cases and controls. Using a two-tailed test and assuming a 90% follow-up (85% power, 5% alpha risk), we estimated that 36 patients with IA and 36 controls were to be needed to detect a difference between the groups. We employed either the Mann-Whitney *U*-test or Student *t* test for continuous data, and the chi-squared test (Yates correction, if needed) for categorical data. Diagnostic performance for different cytokine values are expressed as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), and positive and negative likelihood ratio. The area under receiver operating characteristic (ROC) curve will be constructed to assess how changes in the various markers altered sensitivity and 1 minus specificity.

A second part of the study analyzed only patients with IA. We performed univariate analyses that included clinical variables associated with an increased risk of mortality and different cytokine concentrations studied on day 0. A Cox proportional hazards model to identify independent variables associated with mortality was also done. Variables that were found to have a significance level of  $P < 0.25$  were included in the multivariate Cox regression model. Variables that were moderately/highly correlated ( $r > |0.5|$ ) were excluded from the multivariate Cox regression model to avoid multiplicity problems. We then performed a final selection of the variables associated with mortality at six weeks applying the forward stepwise selection method [ $p_{in} < 0.05$ ,  $p_{out} < 0.10$ ], for which the likelihood ratio was used. All statistical calculations were performed using the Statistical Package for the Social Sciences (Version SPSS 19.0) for Windows. Statistical significance was established at  $\alpha = 0.05$ . All reported *P*-values were two-tailed.

## Results

### Baseline, diagnostic and therapeutic characteristics

During the study period, we included 36 cases of IA in patients with AL and/or allogeneic HSCT recipients, as well as corresponding controls. The control population was matched by age, baseline disease, treatment strategy, day of treatment strategy initiation, and center. Table 1 summarizes the main characteristics of aspergillosis episodes. The median age of cases was 57.5 (IQR 47–66.8) years. In this group, 55.6% were males; 29 (80.6%) presented neutropenia; and 19 (52.8%) had received corticosteroid therapy. With respect to *Aspergillus* diagnosis, all patients had a compatible CT scan, with most (91.7%) having a positive galactomannan. Of the 36 cases, 15 (41.7%) had a positive culture. Pulmonary infection was diagnosed in 31 (86.1%) patients, with most (31, 86.1%) cases meeting criteria for probable aspergillosis. Voriconazole-containing regimen and amphotericin-containing regimen were used as primary antifungal therapy in 25 (69.4%) and 22 (61.1%) cases, respectively; antifungal combination therapy was used in 23 (63.9%) of these patients.

### Baseline cytokines in both patients with IA and controls

Detectable serum cytokine levels were obtained for IL6, IL10, IL17, and IL 23. IL6 and IL10 cytokine levels on day 0

**Table 1.** Patients' baseline characteristics, clinical features, and diagnosis.

Characteristic	Cases <i>n</i> = 36 (%)	Controls <i>n</i> = 36 (%)	<i>P</i> -value
Age, median (IQR)	57.5 (47–66.8)	55.5 (41.8–64.3)	0.982
Male sex	20 (55.6)	17 (47.2)	0.479
Underlying disease			
Acute leukemia	26 (72.2)	24 (66.7)	0.609
Stem cell transplant	14 (38.9)	12 (33.3)	0.620
Neutropenia	29 (80.6)	27 (75.0)	0.647
Corticosteroid therapy	19 (52.8)	17 (47.2)	0.678
Diagnosis*			
Culture†	15 (41.7)	–	–
Galactomannan	33 (91.7)	–	–
Biopsy	6 (16.7)	–	–
Compatible CT scan	36 (100)	–	–
Infection site			
Pulmonary only	31 (86.1)	–	–
Disseminated IA	5 (13.9)	–	–
Type of IA			
Proven	5 (13.9)	–	–
Probable	31 (86.1)	–	–
Primary antifungal therapy‡			
Voriconazole monotherapy	9 (25)	–	–
Voriconazole-containing regimen	25 (69.4)	–	–
Voriconazole plus amphotericin B	12 (33.3)	–	–
Voriconazole plus echinocandins	4 (11.1)	–	–
Amphotericin B monotherapy	3 (8.3)	–	–
Amphotericin B plus echinocandins	6 (16.7)	–	–
Amphotericin B-containing regimen#	22 (61.1)	–	–
Itraconazole	1 (2.8)	–	–
Combination therapy^	23 (63.9)	–	–
Overall mortality (6 weeks)	6 (16.7)	–	–

\*Patients could have > 1 characteristic within a category

†The following *Aspergillus* were isolated (*n* = 15): 5 (33.3%) *A. fumigatus*; 3 (20%) *A. terreus*; 2 *Aspergillus* spp.; 1 (6.7%) *A. flavus*; and 4 (26.7%) with > 1 *Aspergillus* species.

‡Systemic antifungal therapy with anti-*Aspergillus* activity given for at least 5 consecutive days.

#Amphotericin B presentation: liposomal amphotericin B, 19 (86.4%) cases; and lipidic amphotericin B, 3 (13.6%) cases.

^Voriconazole plus amphotericin B, 12 cases; amphotericin B plus echinocandins, 6 cases; voriconazole plus echinocandins, 4 cases; amphotericin B plus posaconazole, 1 case.

significantly increased in cases compared to controls ( $P = 0.001$  and  $P = 0.025$ , respectively). No difference was observed for both IL17 ( $P = 0.854$ ) and IL23 ( $P = 0.445$ ) cytokine levels on day 0. Barely detectable levels of INF-gamma, IL12 and TNF-alpha were found, with no differences between groups. IL4 was undetectable in serum for both cases and controls. Figure 1 and Figure 2 detail cytokine expression for IL6 and IL10, respectively, on day 0 in cases and controls, as well as their evolution over time among cases. Cytokine levels did not differ between patients with and without neutropenia in both groups. Table 2 compares IA episodes regarding whether IL6 was elevated or not at diagnosis. IL6 was more commonly elevated in patients with neutropenia with no other differences between groups.

Table 3 shows the evolution of all serum cytokine levels in cases. There were no statistically significant differences in most of the cytokine levels at the evaluated timepoints. The only exception was IL6, in which an initial increase at day 7 ( $P = 0.012$ ) and later decrease at day 28 ( $P = 0.028$ ) were observed.

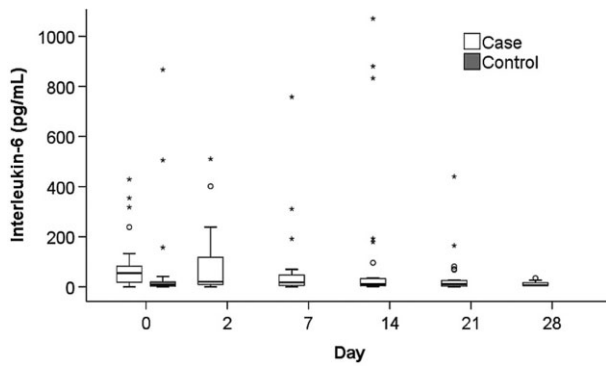
### Baseline cytokines and *Aspergillus* diagnosis

Cytokines statistically related with cases (IL6 and IL10) were evaluated for their ability to predict IA diagnosis on day 0. Table 4 shows the diagnostic sensitivity, specificity, and predictive values for different cutoff values of both cytokines. IL6

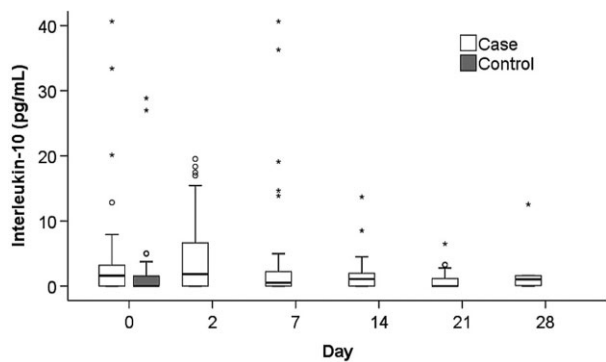
predicted IA with an area under the ROC curve of 0.74 (95% CI 0.62–0.86). The best IL6 cutoff point in predicting IA was 20.85 pg/ml, showing a sensitivity and specificity of 72.2% and 77.8%, respectively; PPV was 76.5% while NPV, 73.7%. IL10 predicted IA with an area under the ROC curve of 0.64 (95% CI 0.51–0.77). The best IL10 cutoff point in predicting IA was 0.045 pg/ml, showing a sensitivity and specificity of 62.9% and 63.9%, respectively; PPV was 62.9% while NPV, 63.9%. When both IL6  $\geq$  20.85 and/or IL10  $\geq$  0.045 were used, sensitivity and NPV increased to 80.6% and 75.0%, respectively; however, specificity decreased to 58.3% and PPV to 65.9%.

### Risk factors for mortality and the impact of baseline cytokines

Among cases, 6-week mortality was 16.7%. The only cytokine related with increased mortality was IL6, with the best cutoff value in predicting mortality being 65.59 pg/ml. In the univariate analysis, IL6 > 65.59 pg/ml was associated with a hazard ratio of 10.58 (95% CI 1.23–90.72) for mortality. Figure 3 shows Kaplan–Meier survival curves, considering patients with a value below or over this IL6 cutoff point. Additionally, IL6 levels decreased over time in survivors ( $P$ -value for trend = 0.037), while remaining stable in those patients who later died ( $P = 0.673$ ). In the multivariable analysis, however, IL6 value was not independently associated with



**Figure 1.** Baseline IL6 expression in cases and controls, and evolution over time among cases. In each box plot, the median value is indicated by the center horizontal line, and the 25th and 75th percentiles are indicated by the lower and upper box horizontal lines. Whiskers above and below the box indicate the 90th and 10th percentiles. Circles and asterisks represent outliers and extreme values, respectively. Mann-Whitney U test indicates that the difference between the case and control groups at day 0 is significant ( $P = 0.001$ ). In the case group, Friedman test indicates that there is a significant difference among 28 days ( $P = 0.037$ ); Wilcoxon test indicates significant changes at day 7 and 28 compared with day 0 (day 7  $P = 0.012$ ; day 28  $P = 0.028$ ), whilst non-significant changes were shown at day 2, 14 and 21 compared with day 0 (day 2  $P = 0.45$ ; day 14  $P = 0.40$ ; day 21  $P = 0.11$ ).



**Figure 2.** Baseline IL10 expression in cases and controls, and evolution over time among cases. In each box plot, the median value is indicated by the center horizontal line, and the 25th and 75th percentiles are indicated by the lower and upper box horizontal lines. Whiskers above and below the box indicate the 90th and 10th percentiles. Circles and asterisks represent outliers and extreme values, respectively. Mann-Whitney U test indicates that the difference between the case and control groups at day 0 is significant ( $P = 0.025$ ). In the case group, Friedman test indicates that there is a significant difference among 28 days ( $P = 0.028$ ); but non-significant changes were shown at each time-point compared with day 0 (Wilcoxon test: day 2  $P = 0.88$ ; day 7  $P = 0.91$ ; day 14  $P = 0.28$ ; day 21  $P = 0.084$ ; day 28  $P = 0.35$ ).

mortality. Independent risk factors for mortality included disseminated aspergillosis (HR 22.52, 95% CI 3.89–130.48) and ICU admission (HR 7.68, 95% CI 1.54–38.38). The area under the ROC curve for the model was 0.94 (95% CI 0.87–1.00), showing an excellent ability to predict mortality.

## Discussion

In this study, we demonstrate that patients with IA—even those with neutropenia—have a differential plasma expression of cytokines compared to those without IA. As a result, real-life quantification of cytokines could serve as a support tool in diagnosing infections in a clinical setting. We also de-

scribe that IL-6 at diagnosis is a marker for more severe patients. Moreover, there is a relationship between the evolution of these cytokines and the prognosis of the infection. This fact is important because it provides objective information regarding the evolution of complex patients with difficult to assess infections.

Establishing a more prompt diagnosis in patients with IA remains challenging.<sup>9</sup> In a previous study<sup>10</sup> that included patients with highly varying immune status, we documented a strong relationship between IA admissions in Barcelona (Spain) and high spore counts in the environment from within 28–42 days before IA diagnosis. This data highlights that interaction between *Aspergillus* and the host begins several days prior to a diagnosis of this infection. As part of a diagnostic approach to IA, cytokine determination in a non-invasive sample such as plasma may be a good marker in identifying patients in whom an inflammatory activation process has begun. Future studies should assess whether an increase in cytokines is earlier than that in other particular biomarkers. Furthermore, future studies should evaluate if an undetectable cytokine profile may help to identify false negative or positive results from galactomannan assays or other fungal diagnostic tools.

IL6 and IL10 constituted the cytokines found to be elevated in plasma at the time of IA diagnosis. This finding is reasonable, given that an initial step in the host response to fungi is the release of pro-inflammatory and anti-inflammatory cytokines by Th1 and Th2 cells,<sup>4,5</sup> with IL6 and IL10 being the most prominent, respectively. It is understandable that IL17 and IL23 could not be detected in plasma, as these cytokines are stimulated in patients' mucosa after host recognition of the fungus.

Prior studies have documented a relationship between the risk of developing IA and cytokine-specific genetic variants, including IL6 and IL10, and their potential qualitative differences in biological function.<sup>11,12</sup> Additionally, investigators have described a quantitative association of elevated levels of different cytokines in serum and bronchoalveolar lavage among a few numbers of patients with IA. Heldt et al. reported high levels of IL6 and IL8 in serum and bronchoalveolar fluid in 10 patients with hematologic malignancies and IA.<sup>13</sup> Gonçalves et al. showed a relationship between IA diagnosis and increased levels of IL1, IL6, IL8, IL17A, IL23, and TNF-alpha in bronchoalveolar lavage from a mixed population of high-risk patients.<sup>14</sup> According to these investigators, IL8 levels higher than 904 pg/ml were identified as the best cutoff level for diagnosis. Finally, Liu et al. detailed increased levels of IL6 and IL8 in both serum and bronchoalveolar lavage in patients with chronic obstructive pulmonary disease (COPD) and IA, compared to those with COPD yet no infection.<sup>15</sup>

In our study, high levels of IL6 at diagnosis identified the most severe patients. This information may help to categorize high-risk patients and provide a more personalized approach. In the same vein, these insights could also support optimal group matching in trials.

This study has several limitations. First, it is plausible that personal intervariability exists among cytokine expression. Second, the impact on cytokine expression due to baseline diseases, different therapies, or concomitant complications, such as mucositis, graft-versus-host-disease or potential co-infections, remains unknown. It remains unclear whether the changes of these cytokines are specific for aspergillosis.



**Table 2.** Characteristics of the cases regarding IL6 elevation at diagnosis.

Characteristic	IL6 elevated <i>n</i> = 20 (%)	IL6 not elevated <i>n</i> = 16 (%)	<i>P</i> -value
Male Sex	11 (55.0)	9 (56.3)	0.940
Acute leukemia	17 (85.0)	9 (56.3)	0.056
Stem cell transplant	10 (50.0)	4 (25.0)	0.252
Neutropenia	19 (95.0)	10 (62.5)	0.030
Corticosteroid therapy	7 (35.0)	12 (75.0)	0.343
Positive culture	6 (33.3)	9 (56.3)	0.179
Positive galactomannan	19 (95.0)	14 (87.5)	0.574
Infection site			
Pulmonary only	17 (85.0)	14 (87.5)	1.000
Disseminated IA	3 (15.0)	2 (12.5)	1.000
Type of IA			
Proven	1 (5.0)	4 (25.0)	0.149
Probable	19 (95.0)	12 (75.0)	0.149
Overall mortality (6 weeks)	5 (25.0)	1 (6.3)	0.196

**Table 3.** Evolution of cytokine serum levels over time among cases.

	Day 0		Day 2		Day 7		Day 14		Day 21		Day 28	
	Mean (SD)	Mean (SD)	<i>P</i> -value*	Mean (SD)	<i>P</i> -value*	Mean (SD)	<i>P</i> -value*	Mean (SD)	<i>P</i> -value*	Mean (SD)	<i>p</i> -value*	
IL4 (pg/ml)	0	0	1.000	0.1 (0.3)	0.180	0 (0.1)	0.317	0	1.000	0.01	0.317	
IL6 (pg/ml)	146.5 (423.9)	156.6 (464.2)	0.447	191.5 (792.1)	<b>0.012</b>	124.3 (289.6)	0.399	307.1 (1397.9)	0.107	12.6 (12.4)	<b>0.028</b>	
IL10 (pg/ml)	4.6 (9.2)	4.6 (6.5)	0.884	4.5 (9.8)	0.910	1.9 (3.0)	0.277	22.1 (108.2)	0.084	2.7 (4.9)	0.345	
IL12 (pg/ml)	0.1 (0.4)	0.1 (0.1)	0.285	0.1 (0.2)	0.593	0	0.180	0.1 (0.3)	0.715	0.1 (0.2)	0.655	
IL17 (pg/ml)	1.9 (4.2)	1.4 (2.9)	0.391	3.0 (9.0)	0.657	2.9 (9.6)	0.615	2.8 (9.7)	0.485	2.6 (6.3)	0.463	
IL23 (pg/ml)	4.4 (6.0)	3.5 (5.9)	0.356	4.0 (5.8)	0.717	4.4 (6.0)	0.679	3.7 (5.1)	0.917	3.7 (6.2)	1.000	
INF-gamma (pg/ml)	0.1 (0.3)	0.2 (0.6)	0.144	0.1 (0.3)	1.000	0.2 (0.4)	0.138	0.2 (0.6)	0.197	0.4 (1.2)	1.000	
TNF-alpha (pg/ml)	0.1 (0.2)	0.2 (0.4)	0.233	0.1 (0.3)	0.799	0.1 (0.5)	0.593	0.1 (0.4)	0.500	0.1 (0.2)	1.000	

\*All comparison and *P*-values are calculated per day 0 cytokine values. Abbreviations. SD: standard deviation. Significant *P*-values are showed in bold.

**Table 4.** Diagnostic performance of different cutoff values of IL6 and IL10.

Cytokine value (pg/ml)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
IL6 ≥ 4.04	91.7	30.6	56.9	78.6
IL6 ≥ 5.87	86.1	36.1	57.4	72.2
IL6 ≥ 7.393	80.6	38.9	56.9	66.7
IL6 ≥ 20.85	72.2	77.8	76.5	73.7
IL6 ≥ 26.428	61.1	80.6	75.9	67.4
IL6 ≥ 57.042	47.2	88.9	80.9	62.7
IL6 ≥ 80.186	27.8	88.9	71.4	55.2
IL6 ≥ 294.3	11.1	91.7	57.1	50.8
IL10 ≥ 0.045	62.9	63.9	62.9	63.9
IL10 ≥ 1.366	51.4	72.2	64.3	60.5
IL10 ≥ 2.148	40.0	80.6	66.7	58.0
IL10 ≥ 2.59	34.3	83.3	66.7	56.6
IL10 ≥ 3.21	28.6	86.1	66.7	53.4
IL10 ≥ 7.74	12.3	94.4	71.4	53.1
IL6 ≥ 20.85 and/or IL10 ≥ 0.045	80.6	58.3	65.9	75.0

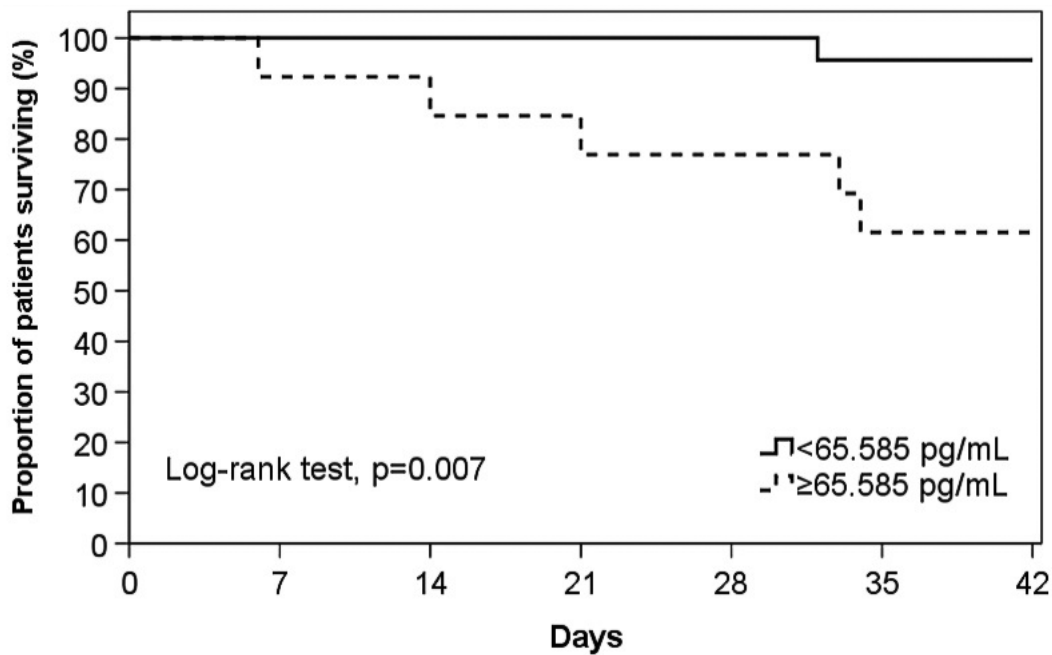
In contrast, our strengths include a description of the largest series of patients with IA in whom cytokines have been prospectively collected. These patients shared homogenous characteristics, and the comparator group was matched on the most important factors.

To conclude, this paper shows that cytokine levels measured in plasma may be used as a support tool, alongside other cur-

rent tests, in diagnosing IA. High levels of IL6 at IA diagnosis are related with worse outcomes.

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**Figure 3.** Kaplan–Meier survival curves considering the best IL6 cutoff value to predict mortality.

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### Declaration of interest

PP-A has received honoraria for talks on behalf of Merck Sharp and Dohme, Gilead, Lilly, ViiV Healthcare, and Gilead Science. P.B. has received honoraria from Amgen, BMS, Gilead, Incyte, Jazz Pharmaceuticals, Miltenyi biotech, Novartis, and Pfizer. CG-V has received honoraria for talks on behalf of Gilead Science, MSD, Novartis, Pfizer, Janssen, Lilly as well as a grant from Gilead Science and MSD. The other authors have no conflicts of interest to declare.

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