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Cytokine profiles linked to fatal outcome in infective prosthetic valve endocarditis

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Infective endocarditis is a disease normally of bacterial cause which affects the endocardic tissue, specifically the valves (native or prosthetic). It is a serious illness and mortality rates remain high, ranging between 20% and 40%. Previous reports have evidenced the potential role of cytokines in the diagnosis of this disease, but no information is available on their relationship with outcome. We recruited 26 consecutive patients with late prosthetic valve endocarditis requiring surgical treatment according to Duke criteria. Eight cytokines were measured in plasma in the first 24 h following diagnosis by using a Bio-Rad multiplex assay. Levels of IL-6, IL-8 and interferon gamma (IFN-γ) were higher in non survivors. Receiver operating characteristic curve analysis evidenced that IL-6, IL-8 and IFN-γ behaved as good diagnostic tests for identifying those patients with fatal outcome (area under the curve, CI 95%, p): IL-6: [0.81 (0.61–1.00) 0.012]; IL-8 [0.76 (0.56–0.96) 0.035]; IFN-γ [0.79 (0.59–0.99) 0.021]. Levels of IL-6, IL-8 and IFN-γ correlated positively between them, indicating that they are produced as consequence of a simultaneous response to the infection. Our findings support the participation of IL-6, IL-8 and IFN-γ in the events linked to fatal outcome in infective prosthetic valve endocarditis.

Key words: Infective; endocarditis; cytokines; prosthetic; mortality.

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Infective endocarditis is a disease normally of bacterial cause which affects the endocardic tissue, specifically the valves (native or prosthetic). It is a serious illness and despite the improvements in antimicrobial and surgical treatment, as well as in diagnosis, mortality rates remain high, ranging between 20% and 40% according to the different series (1). Works from several groups support the value of inflammatory markers for the early diagnosis of infective endocarditis (being proposed as a complement to the Duke's diagnosis criteria), for monitoring disease evolution as well as response to treatment (2). The clinical profile of patients has changed in the last decades in the same way the disease has changed. Currently, the percentage of patients with endocarditis and negative blood cultures has significantly increased due to the frequent use of broad spectrum antibiotics, which complicates both diagnosis and antibiogram guided selection of the correct/specific antibiotic, therefore worsening the prognosis. Identifying those patients with infective endocarditis at risk of suffering fatal outcome could help to individualize treatment in

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this disease. Here, we measured levels of eight major Th1 and Th2 cytokines in plasma from patients with prosthetic valve endocarditis, by using a multiplex approach, and studied their relationship with mortality.

MATERIALS AND METHODS

Patients and samples

Twenty-six consecutive patients with late prosthetic valve endocarditis requiring surgical treatment were recruited according to Duke criteria. Approval for the study protocol for both the scientific and the ethical aspects was obtained from the Scientific Committee for Clinical Research of our Hospital. Informed consent was obtained directly from each patient/legal representative before enrolment. All the patients underwent an exhaustive clinical study which included transthoracic and transoesophageal echocardiography. Blood cultures were taken at the time of admission. In addition, 5 mL of blood was collected in the first 24 h following the diagnosis of endocarditis by using EDTA tubes. Plasma was obtained after proper centrifugation, and stored at −80 °C until cytokine evaluation.

Cytokines profiling

Cytokines were measured by using a 8 plex kit purchased to Bio-Rad (Hercules, CA, USA), including IL-2, IL-4, IL-6, IL-8, IL-10, interferon gamma (IFN-γ), granulocyte macrophage colony-stimulating factor (GMCSF), and tumour necrosis factor alpha (TNF-α). Cytokine profiling was performed on a Luminex platform. Limits of detection were as follows (in pg/mL): IL-2 (5.1), IL-4 (0.3), IL-6 (5.2), IL-8 (1.9), IL-10 (3.2), IFN-γ (1.6), GMCSF (2.6) and TNF-α (6.4). Cytokine values less than the level of detection were reported as being equal to the level of detection. Mann–Whitney *U*-test was employed to assess potential differences in cytokine median levels between survivors and patients with fatal outcome. The accuracy values of the cytokines analysed for diagnosing mortality were studied by calculating areas under the receiver operating characteristic curve. Correlation between cytokines was assessed by using the Spearman Karber test. Significance was fixed at the level ($p < 0.05$). Data analysis was performed using SPSS for Windows version 20.0 software (IBM-SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Of patients, 65.4% (n = 17) were men. Patients were 64.92 ± 13.37 years old (mean, SD; range 39–89 years). The main risk factors for developing endocarditis were antecedent of dental procedures [73.1% of the cases (n = 19)], diabetes mellitus [26.9% (n = 7)], chronic renal failure [23.1% (n = 6)] and immune deficiency [23.1% (n = 6)]. Absence of any identifiable precedent was found in 38.5% of the cases. The main symptoms were fever, present in 17 patients (65.4%) and heart failure [n = 11 (42.3%)]. Other signs were heart murmur, [n = 9 (34.6%)], Roth spots and Splinter haemorrhage [n = 3 (11.5%)]. Heart failure was the main indication for surgical treatment (76.92%). Eight patients died early following surgery (≤30 days) due to septic multi-organ failure (n: 5) or myocardial failure (n: 3). This mortality rate (30.77%) is similar to that observed by other authors. The most frequent causative microorganisms were Staphylococci spp. (Methicillin-sensible *Staphylococcus aureus* n = 6, Methicillin-resistant *Staphylococcus aureus* n = 2, coagulase-negative Staphylococci, n = 7), Streptococci spp. n = 3 and Enterococci spp. n = 3, being five cases negative in the cultures. Characteristics of non survivors are showed in Table 1.

Patients with detectable levels of IL-2, IL-10, TNF-α, IL-4, IL-6, IL-8, GM-CSF and IFN-γ were: n = 2, 1, 2, 7, 10, 15, 8 and 15 respectively. Levels of IL-6, IL-8 and IFN-γ were higher in non survivors than in survivors ($p < 0.05$; Fig. 1). Receiver operating characteristic curve analysis evidenced that IL-6, IL-8 and IFN-γ behaved as good diagnostic tests for identifying those patients with fatal outcome [area under the curve, CI 95%, p]: IL-6: [0.81 (0.61–1.00) 0.012]; IL-8 [0.76 (0.56–0.96) 0.035]; IFN-γ [0.79 (0.59–0.99) 0.021] (Fig. 2). Levels of IL-6, IL-8 and IFN-γ correlated positively between them, indicating that they are produced as consequence of a simultaneous and prompt response to the infection (cytokines, correlation coefficient): (IL-6, IL-8: 0.730; IL-6, IFN-γ: 0.375; IL-8, IFN-γ: 0.505; $p < 0.05$).

Table 1. Clinical characteristics of non survivors

Sex	Age	Cytokine levels in plasma			Infecting microbe
		IL-6 (pg/mL)	IL-8 (pg/mL)	IFN-γ (pg/mL)	
Male	62	54	331	207	Enterococci spp.
Male	81	4617	23	253	Staphylococci spp.
Male	54	35748	651	3099	Streptococci spp.
Male	71	4	2	2	Staphylococci spp.
Male	74	360	17	1001	Enterococci spp.
Female	69	103	13	81	Staphylococci spp.
Male	80	4	12	770	Staphylococci spp.
Male	89	78	20	3099	Staphylococci spp.

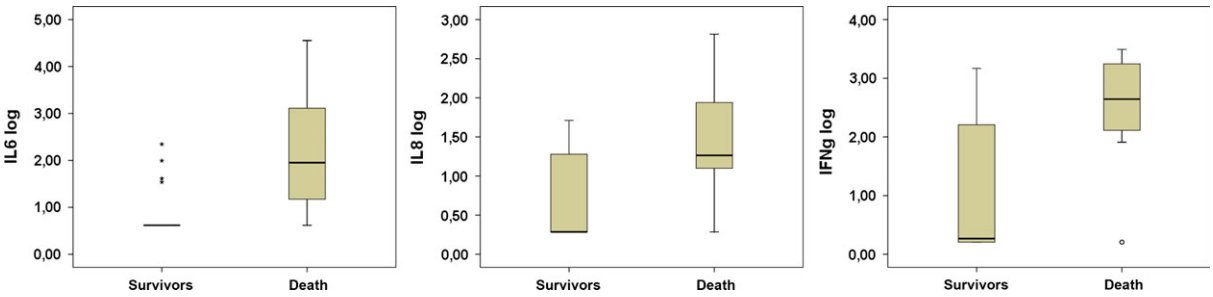

Fig. 1. Box plots showing cytokine levels in survivors and non survivors.

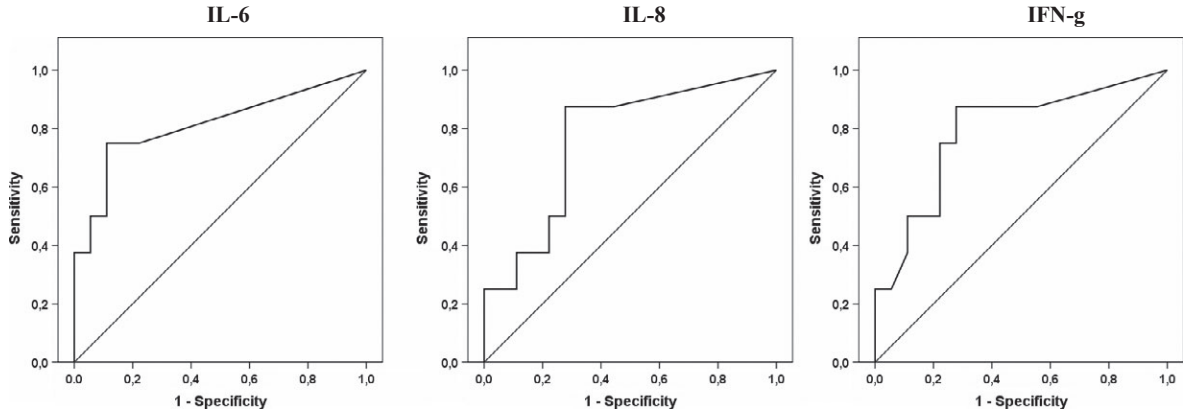

Fig. 2. Receiver operating characteristic curves of cytokines for mortality.

IL-6, IL-8, IFN-γ are pro-inflammatory cytokines participating in the development of the innate immunity, and have been described to be associated with bad outcome in severe infectious conditions, such as sepsis and severe respiratory illness caused by influenza (3, 4). On the contrary, available information on the role of these cytokines in endocarditis is scarce, and most of the reports are focused on their potential use in the diagnosis of the disease. Alter et al., Watkin et al. and Rawczynska-Englert I et al. had already described elevated levels of IL-6 in infective endocarditis, and propose a potential diagnostic use of this cytokine (2, 5, 6). Ekdahl et al. proposed IL-8-containing cells in infected heart valves as a marker of disease activity (7). As far as we know, there are no reports evaluating the participation of IFN-γ in this condition. An exacerbated inflammatory response driven by IL-6, IL-8 and IFN-γ could translate into tissue damage at the valvular level. In addition, high systemic levels of these cytokines could reflect a poor control of the infection in the early moments of the disease in non survivors. Systematic evaluation of immune status in patients with endocarditis could help to confirm this hypothesis.

Conclusions

Our findings support for the first time the participation of IL-6, IL-8 and IFN-γ in the events linked to fatal outcome in infective prosthetic valve endocarditis. Measuring levels of these cytokines could help to individualize treatment in this disease, by identifying those patients at risk for worst outcomes. These results could help to better stratify patients in clinical trials on endocarditis, by better assessing their initial risk of mortality. Further studies should confirm their potential clinical value as prognostic biomarkers in this disease.

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