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Inflammatory biomarkers in infective endocarditis: machine learning to predict mortality

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Summary

Infective endocarditis (IE) is the cardiac disease with the highest rates of mortality. New biomarkers that are able to identify patients at risk for death are required to improve patient management and outcome. This study aims to investigate if cytokines, chemokines and growth factors measured at IE diagnosis can predict mortality. Patients with definite IE, according to the Duke's modified criteria, were included. Using high-performance Luminex assay, 27 different cytokines, chemokines and growth factors were analyzed. Machine learning techniques were used for the prediction of death and subsequently creating a decision tree, in which the cytokines, chemokines and growth factors were analyzed together with C-reactive protein (CRP). Sixty-nine patients were included, 41 (59%) male, median age 54 [interquartile range (IQR) = 41–65 years] and median time between onset of the symptoms and diagnosis was 12 days (IQR = 5–30 days). The in-hospital mortality was 26% (n = 18). Proinflammatory cytokines interkeukin (IL)-15 and C-C motif chemokine ligand (CCL4) were found to predict death, adding value to CRP levels. The decision tree predicted correctly the outcome of 91% of the patients at hospital admission. The high-risk group, defined as CRP ≥ 72 mg/dL, IL-15 ≥ 5·6 fg/ml and CCL4 ≥ 6·35 fg/ml had an 88% in-hospital mortality rate, whereas the patients classified as low-risk had a mortality rate of 8% (P = < 0·001). Cytokines IL-15 and CCL4 were predictors of mortality in IE, adding prognostic value beyond that provided by CRP levels. Assessment of cytokines has potential value for clinical risk stratification and monitoring in IE patients.

Keywords: biomarkers, cytokines, infective endocarditis, mortality

Introduction

Infective endocarditis (IE) is a disease with remarkably high mortality and morbidity. The in-hospital mortality ranges from 15 to 30% [1–6] and other complications, such as embolic events, heart failure and septic shock, are often seen [7]. Because of the many different aspects of the disease, prediction of outcome continues to be a challenge. Early detection of patients at high risk for death may optimize patient management, including early surgery with a possible positive effect on the prognosis of the patient [8–10].

Currently, assuming appropriate antibiotic usage, there are four main factors that are used for the risk stratification of IE: echocardiographic findings, patient characteristics, the presence of cardiac or non-cardiac complications and the infective organism [11]. However, finding new prognostic factors can be an essential step in already improving the early risk stratification in an attempt to identify complicated IE patients at diagnosis.

Studies that evaluated the biomarker C-reactive protein (CRP) of IE patients at hospital admission showed that CRP is an independent predictor of poor clinical outcome [12–16]. However, CRP is a non-specific biomarker and may be elevated in other infections, non-infectious inflammatory disorders and neoplasms. Cytokines are involved in IE pathogenesis, and therefore may play an important

role in early risk stratification. Finding associations between cytokine levels and clinical outcome may provide new opportunities for early detection of complicated IE. This may support the decision of the endocarditis team for either more aggressive treatment or early surgery.

Cytokines are small proteins that are secreted by cells to establish a specific effect on the interaction and communication between cells [17]. Activated macrophages produce proinflammatory cytokines that are responsible for the up-regulation of inflammatory reactions, whereas regulatory/anti-inflammatory cytokines regulate this response [18]. Chemotactic cytokines (chemokines) are responsible for the activation and migration of leukocytes to the site of infection [18]. Growth factors are biologically active cytokines or proteins that can promote or inhibit cellular growth [19]. Lower levels of growth factors are often seen during systemic inflammation, which may affect the healing process [20].

Previous studies have demonstrated that cytokine patterns of IE patients differ from those of healthy individuals [20–28]. Even when IE is compared to other non-IE infections, the cytokine profile is different [24,25,27,28], which suggests a specific cytokine profile in the IE setting. Subsequently, when comparing the cytokine levels of IE patients with the clinical outcome, higher levels of cytokines were found in IE patients who died [26,27]. However, one of these studies included only 26 subjects: late prosthetic IE patients who had already required surgery, thus representing a specific IE population. In addition, both studies analyzed only a small number of cytokines. Therefore, the present study was designed to analyze 27 different cytokines, chemokines and growth factors to assess a powerful risk prediction model in patients with IE. Indeed, this large number of cytokines has never been evaluated before in this setting.

The aim of this study is to determine if cytokines, chemokines and growth factors assessed at IE diagnosis can predict in-hospital death. Additionally, we aimed to improve our understanding of the immunopathological pathways in IE.

Methods

Study population

Patients with definite IE, according to the Duke's modified criteria, consecutively admitted to the University Hospital, Federal University of Minas Gerais, Brazil between February 2012 and February 2018, were included in this prospective cohort study. Exclusion criteria were the use of antibiotics for more than 1 week prior to collecting the blood samples and patients who died or underwent cardiac surgery before collecting the blood samples. Patients were treated according to the latest available European Society of Cardiology (ESC) IE guidelines [10]. The study was approved by the institution's medical ethics committee, and all participants gave written informed consent obtained at hospital admission.

Patient characteristics were obtained at hospital admission and included age, gender, predisposing cardiac conditions, the presence of chronic renal disease and diabetes mellitus and clinical and echocardiographic findings. In addition, the causative microorganism was determined. Follow-up data of the patients were obtained until hospital discharge or occurrence of death. The outcome of this study was death during treatment of IE, caused by any complication of IE or its treatment.

Biomarkers measurement

High performance Luminex 27-plex assay (Bio-Rad, Hercules, CA, USA) was used for the simultaneous detection and quantification of various cytokines and growth factors, including: C-X-C motif chemokine ligand (CXCL) chemokines – CXCL8 [interleukin (IL)-8] and CXCL10 [IFN-inducible protein-10 (IP-10)]; C-C motif chemokine ligand (CCL) chemokines – CCL11 (eotaxin), CCL3 [macrophage inflammatory protein 1-α (MIP-1α)], CCL4 (MIP-1β), CCL2 [monocyte chemotactic protein-1 (MCP-1)], CCL5 [regulated on activation, normal T cell expressed and secreted (RANTES)]; interleukins IL-1β, IL-6, tumor necrosis factor (TNF)-α, IL-12, IFN-γ, IL-17, IL-1 receptor antagonist (IL-1Ra), IL-2, IL-4, IL-5, IL-7, IL-9, IL-10, IL-13, IL-15; growth and colony-stimulating factors – basic fibroblast growth factor (FGF-basic), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF).

Twofold diluted patient serum samples were analyzed according to the manufacturer's instructions. Various reagents were used, including plates and standards. A minimum of 50 beads was acquired per biomarker on a Bio-Plex 200 instrument (Bio-Rad). The median fluorescence intensity was determined for each biomarker using Luminex xPONENT software version 3.1 (Merck Millipore, Billerica, CA, USA). Standard curves were generated for each biomarker using fourfold serial dilutions of manufacturer-provided premixed lyophilized standards. The biomarkers were able to be measured with a sensitivity of fg/ml. The biomarker concentrations were calculated based on the standard curve acquired with Bio-Plex Manager software version 6.1 (Bio-Rad) using a 5th-parameter logistic fit curve.

Data analysis

We used non-parametric tests to compare variables among subgroups, and a two-sided *P*-value less than

or equal to 5% was considered significant. Continuous variables were compared using the Mann–Whitney *U*-test, while categorical variables were compared using the Fisher's exact test with mid-*P* adjustment [29]. To estimate risk ratio confidence intervals (CI), we used Taylor's series approximation. Classification trees (CART) were used to determine the thresholds of each variable to predict mortality and to create a multivariate algorithm to classify patients according to their mortality risk. CART is a supervised machine learning method and the thresholds of the variables that minimize the sum of the Gini impurity within subgroups are obtained [30]. Gini impurity is a measure of statistical dispersion which has a value of zero when all cases in a group have the same measured outcome and an increasing value as the group becomes more heterogeneous, with a maximum value of 1 [30]. For example, in this study a subgroup would have a Gini impurity value of zero if all patients survived or if all patients died within it. Thresholds were obtained allowing a split in a variable only if a minimum reduction of 0·01 of the Gini impurity (sum within subgroups) was achieved and requiring a minimum size of eight patients in each subgroup, to avoid overfitting. In univariate analysis, only one split was permitted for each variable. All calculations were performed using the software R version 3.5.1 [31]. The software has several packages related to different functionalities. Decision trees were built using the package 'rpart' [32] and the multivariate tree was pruned after cross-validation. Risk ratio confidence intervals were determined using the package 'fmsb' [33].

Results

Baseline characteristics

Two hundred and twenty-six patients were eligible for inclusion in the study. Initially, 76 patients with definite IE according to the Duke's modified criteria were selected. However, we could not collect blood samples from four patients and the clinical data of two other patients. One patient was removed from the cohort due to misdiagnosis. The final study cohort therefore comprised 69 patients.

Twenty-eight patients were female (41%), the median age was 54 years [interquartile range (IQR) = 42–64 years]. The median time between onset of the symptoms and the diagnosis of IE was 12 days (IQR = 5–30 days). After IE diagnosis, the median duration of hospitalization was 44 days (IQR = 30–57 days).

The most common clinical findings were fever (n = 56, 81%), heart murmur (n = 52, 75%), anorexia (n = 42, 61%), weight loss (n = 36, 52%) and myalgia (n = 11, 16%). Classical specific findings of IE were rare and consisted of Janeway lesions (n = 4, 6%), splinter hemorrhages (n = 4, 6%), conjunctival petechiae (n = 2, 3%) and Osler nodes (n = 2, 3%). Rheumatic heart disease was the most common predisposing condition and was present in 18 patients (26%), whereas chronic renal disease was the most common co-morbidity and was found in 13 patients (19%).

Vegetation was found in 94% of the echocardiograms, with a median length of 8 mm (IQR = 7–15 mm). Left-sided native valve endocarditis (NVE) was diagnosed in 39 patients (56%): 16 patients (23%) had aortic valve involvement and 23 (33%) had mitral valve involvement. Right-sided IE was diagnosed in 16 patients (23%) and its primary cause was device-related IE, including pacemaker and implantable cardioverter-defibrillator (n = 11, 69% of total right-sided IE). Prosthetic valve endocarditis (PVE) occurred in 10 patients (14%).

The main causative microorganisms of IE were staphylococci (n = 25, 36%). Coagulase-negative staphylococci accounted for 19% (nine PVE and four NVE) and *Staphylococcus aureus* for 17% (nine NVE and three PVE) of IE cases. Streptococci were found as a cause in nine patients (13%). Culture results also included Gram-negative bacteria (n = 5, 7%), polymicrobial infection (n = 3, 4%) and fungi (n = 2, 3%). In 21 patients (30%) a causative microorganism could not be identified with routine cultures, and they were considered as culture-negative IE.

Comparison of the baseline characteristics between patients who died and those who survived can be found in Table 1.

Complications and outcomes

Thirty-seven patients (54%) were diagnosed with heart failure at presentation or during treatment. In addition, 10 patients (15%) had an ischemic neurological event, one patient had a hemorrhagic neurological event (1%) and four patients (6%) had an embolic event other than ischemic stroke. Valve regurgitation complications were detected by echocardiogram in 10 patients (14%) after leaflet rupture or perforation. An abscess in the heart was found in two patients (3%) and prosthesis dysfunction in one patient (1%). Thirty-seven patients (54%) were operated, due mainly to severe heart failure (n = 17, 46% of operations). Other surgery indications were uncontrolled infection (n = 9, 24%), device-related IE (n = 8, 22%) and embolic events (n = 3, 8%). The mortality rate was similar between the patients who underwent surgery (24%) compared to those who did not (28%).

Eighteen patients (26%) died during hospitalization. Comparisons of biomarker levels between the subgroups of patients who survived or died are shown in Table 2. Table 3 shows the thresholds found for the laboratory results, vegetation size and biomarkers. Furthermore,

Table 1. Baseline characteristics of the study population stratified according to in-hospital mortality

Baseline characteristics*	Survived (n = 51)	Died (n = 18)	P-value
Age (years)	54 (39–64)	54 (45–64)	0·608
Female	22 (43·1%)	6 (33·3%)	0·487
Diabetes mellitus	3 (5·9%)	5 (27·8%)	0·027
Chronic renal disease	7 (13·7%)	6 (33·3%)	0·091
Predisposing conditions			
Prosthetic valve	14 (28·6%)	4 (22·2%)	0·632
Rheumatic valve disease	12 (23·5%)	6 (33·3%)	0·433
Endocavitary devices (pacemaker/ICD)	11 (21·6%)	3 (16·7%)	0·693
Degenerative valve disease	10 (19·6%)	3 (16·7%)	0·819
Mitral valve prolapse	10 (19·6%)	3 (16·7%)	0·819
Congenital heart disease	4 (7·8%)	0 (0·0%)	0·289
Previous IE	3 (5·9%)	1 (5·6%)	0·988
Heart failure	25 (49·0%)	12 (66·7%)	0·212
Clinical finding			
Fever	44 (86·3%)	12 (66·7%)	0·091
Weight loss	29 (60·4%)	7 (41·2%)	0·188
Anorexia	32 (65·3%)	10 (55·6%)	0·479
Myalgia	7 (13·7%)	4 (22·2%)	0·421
Heart murmur	39 (76·5%)	13 (72·2%)	0·718
Laboratorial findings			
CRP (mg/l)	59 (31–148)	111 (76–235)	0·026
Hemoglobin (g/dl)	10·4 (9·0–11·3)	9·1 (7·7–10·1)	0·028
Leukocytes (cells × 10^6/ml)	10·4 (6·5–13·1)	13·5 (7·0–16·0)	0·199
Platelet count (cells × 10^3/ml)	183 (139–245)	170 (95–205)	0·268
INR	1·2 (1·1–1·4)	1·3 (1·1–1·4)	0·699
Albumin (g/dl)	3·0 (2·8–3·2)	2·8 (2·5–3·1)	0·702
Etiology			
Staphylococcus aureus	9 (17·6%)	3 (16·7%)	0·956
Streptococci	8 (15·7%)	1 (5·6%)	0·310
Coagulase-negative staphylococci	8 (15·7%)	5 (27·8%)	0·288
Enterococci	3 (5·9%)	1 (5·6%)	0·988
Others	7 (13·7%)	3 (16·7%)	0·752
Negative culture	16 (31·4%)	5 (27·8%)	0·798
Location of the infection			
Native aortic valve	11 (21·6%)	5 (27·8%)	0·598
Native mitral valve	16 (31·4%)	7 (38·9%)	0·571
Tricuspid valve	4 (7·8%)	1 (5·6%)	0·818
Prosthetic valve	7 (13·7%)	3 (16·7%)	0·752
Device-related	9 (17·6%)	2 (11·1%)	0·560
Echocardiogram findings			
Vegetation size (mm)	7·7 (5·0–11·1)	15·0 (8·0–20·0)	0·019
Interventions			
Cardiac surgery	28 (54·9%)	9 (50·0%)	0·728

*Continuous variables are expressed as median (interquartile range) and categorical variables are expressed as absolute numbers (percentage).

Table 3 shows the univariate prediction of death using the CART method.

Several predictors of in-hospital mortality of IE patients were found by the univariate analysis, as shown in Table 3. Greater vegetation size in echocardiogram, elevated CRP, leukocytes, CXCL8, CXCL10, CCL2, CCL3, CCL4, IFN-γ, IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-9, IL-10, IL-12P70, IL-15, IL-17A, PDGF, VEGF, G-CSF and GM-CSF, as well as reduced hemoglobin, were significantly associated with in-hospital death.

The multivariate classification tree and its cross-validation are shown in Figs. 1 and 2. The optimal number of splits was determined minimizing cross-validation (69 subgroups) relative risk. The algorithm predicted correctly the outcome of 63 patients (91%), with a sensitivity of 78% and specificity of 96%. Three groups with low risk of death and one with high risk of death were created. Merging the three groups with low risk of death into a single group, we ended up with two groups: a low-risk group comprising 53 patients

Table 2. Levels of 27 biomarkers (fg/ml) of the patients at baseline stratified according to in-hospital mortality

	Survived (n = 51)	Died (n = 18)	P-value
CXCL chemokines			
CXCL8	3·5 (2·3–7·7)	10·8 (6·0–30·5)	**<0·001**
CXCL10	411·3 (225·5–814·0)	647·8 (472·9–1245·4)	0·066
CCL chemokines			
CCL2	9·0 (6·7–15·8)	19·2 (13·2–27·7)	**0·004**
CCL3	1·0 (0·7–2·0)	1·5 (0·7–3·2)	0·328
CCL4	5·4 (3·7–8·5)	11·9 (7·3–14·9)	**<0·001**
CCL5	67·3 (51·8–92·1)	59·7 (41·0–113·4)	0·832
CCL11	14·2 (8·3–21·4)	16·8 (13·6–30·0)	0·128
Interleukins			
IFN-γ	22·2 (17·4–31·0)	33·6 (17·6–39·5)	0·077
TNF-α	14·7 (10·6–29·6)	18·0 (13·7–42·6)	0·385
IL-1β	0·9 (0·6–1·1)	1·0 (0·7–1·2)	0·389
IL-1RA	31·9 (25·2–52·8)	70·9 (33·6–118·0)	0·047
IL-2	0·7 (0·5–1·5)	1·3 (0·7–4·9)	0·097
IL-4	0·33 (0·28–0·42)	0·41 (0·31–0·66)	0·191
IL-5	2·5 (1·8–3·8)	1·9 (1·3–3·2)	0·134
IL-6	3·6 (1·4–12·6)	17·0 (5·8–48·1)	<0·001
IL-7	1·9 (1·3– 2·7)	2·5 (1·5–2·7)	0·225
IL-9	6·8 (4·9–9·5)	11·0 (6·3–18·0)	0·048
IL-10	3·0 (1·9–8·2)	7·3 (4·1–14·2)	0·016
IL-12P70	2·1 (1·6–6·3)	4·7 (2·2–9·3)	0·077
IL-13	0·8 (0·4–1·3)	0·9 (0·4–2·6)	0·828
IL-15	5·5 (3·3–9·2)	11·6 (8·2–19·1)	<0·001
IL-17A	5·4 (3·9–8·1)	7·3 (4·3–13·3)	0·157
Growth factors and colony-stimulating factors			
FGF-basic	4·7 (3·8–6·6)	5·6 (3·9–7·5)	0·370
PDGF	78·3 (37·1–174·3)	99·1 (75·4–142·7)	0·234
VEGF	6·4 (3·0–15·1)	15·4 (7·8–37·1)	0·023
G-CSF	6·1 (3·9–9·4)	18·7 (6·7–33·8)	0·021
GM-CSF	10·1 (8·2–18·3)	13·8 (10·6–36·8)	0·048

Variables are expressed as median (interquartile range). CCL = C-C motif chemokine ligand; CXCL = C-X-C motif chemokine ligand; IFN = interferon; IL = interleukin; FGF = fibroblast growth factor; PDGF = platelet-derived growth factor; VEGF = vascular endothelial growth factor; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor.

(77%) with a death risk of 8% and a high-risk group comprising 16 patients (23%) with a death risk of 88%. The comparisons between these last two groups are shown in Table 4.

The risk subgroups are also illustrated in Fig. 3, showing first- and second-decision nodes in Fig. 3a, involving IL-15 and CRP, while the second- and third-decision nodes are illustrated in Fig. 3b, involving CRP and CCL4.

Long-term follow-up

All patients were followed-up after hospital discharge on an out-patient basis every 4 months or more often according to their clinical status. The overall mean follow-up period was 2·6 years (range = 3·9 months to 6·4 years). Three patients presented with new episodes of IE at 6, 10 and 15 months following discharge. Six patients underwent cardiac surgery and three patients died at a mean follow-up of 3·4 years. During the first 90 days after discharge, no evidence of reinfection or relapse IE was detected and none of the patients died.

Discussion

In our study we found that IL-15, CCL4 and CRP levels are predictors of the outcome of IE patients and created an accurate risk prediction model based on a classification tree. The classification tree predicted the outcome of 91% of the patients at hospital admission correctly. A high-risk group for death was identified with an in-hospital mortality rate of 88%, whereas for those classified as low-risk patients the mortality rate was only 8%.

We used classification trees because they are able to capture complex non-linear interactions among variables while keeping model interpretability, allowing easy incorporation of the results into medical practice [34]. In the multivariate model, the tree is built stepwise and the variable included in each node is the one that results in the greater reduction of Gini impurity. Therefore, we have found the three most important predictors of death in IE respecting our model constraints.

Current factors that determine patient management focus on the progression of the disease, rather than on risk stratification at diagnosis [8]. Early aggressive antibiotic treatment may possibly have a positive impact on the prognosis of the patient. New prognostic markers for risk stratification are therefore required in order to identify patients at risk for complication at an early stage of the disease. We therefore aimed to investigate the role of different cytokines, chemokines and growth factors for early risk stratification in patients with IE. Several cytokines that have never been evaluated previously in this setting were included; among these were chemokines and growth factors. Therefore, our study also expands our knowledge regarding the complex inflammatory response and cytokine profiles in IE.

Existing literature concerning the relationship between cytokines, growth factors and colony-stimulating factors and death in IE is scarce. In previous studies, IL-6, CXCL8 and IFN-γ were described as possible biomarkers for predicting mortality [26,27].

In our study, several biomarkers were significantly associated with death in the univariate model, but only IL-15 and CCL4 remained significant in the multivariable analysis.

Table 3. Thresholds found by classification trees to predict mortality

	Threshold (high risk)	Died in low-risk group	Died in high-risk group	RR (95% CI)	*P*-value
Laboratory findings					
CRP (mg/l)	≥72·0	3/33 (9%)	15/36 (42%)	4·6 (1·5–14·4)	0·002
Hemoglobin (g/ml)	≤8·5	11/58 (19%)	7/11 (64%)	3·4 (1·7–6·7)	0·005
Leukocytes (10^6 cells/ml)	≥13·0	8/46 (17%)	10/23 (44%)	2·5 (1·1–5·5)	0·027
Platelet count (cells × 10^3/ml)	≤180	5/30 (17%)	12/33 (36%)	2·2 (0·9 – 5·5)	0·088
INR	n.a.	n.a.	n.a.	n.a.	n.a.
Albumin (g/dL)	n.a.	n.a.	n.a.	n.a.	n.a.
Echocardiogram findings					
Vegetation size (mm)	≥13·5	8/41 (20%)	9/18 (50%)	2·6 (1·2–5·6)	0·025
CXCL chemokines (fg/ml)					
CXCL8	≥3·7	1/28 (4%)	17/41 (42%)	11·6 (1·6–82·3)	<0·001
CXCL10	≥518·4	5/37 (14%)	13/32 (41%)	3·0 (1·2–7·5)	0·013
CCL chemokines (fg/ml)					
CCL2	≥13·0	4/40 (10%)	13/28 (46%)	4·6 (1·7–12·8)	0·001
CCL3	≥1·6	8/45 (18%)	10/24 (42%)	2·3 (1·1–5·1)	0·041
CCL4	≥10·5	7/50 (14%)	11/19 (58%)	4·1 (1·9–9·1)	<0·001
CCL5	n.a.	n.a.	n.a.	n.a.	n.a.
CCL11	n.a.	n.a.	n.a.	n.a.	n.a.
Interleukins (fg/ml)					
IFN-γ	≥32·4	8/48 (17%)	10/21 (48%)	2·9 (1·3–6·2)	0·011
TNF-α	n.a.	n.a.	n.a.	n.a.	n.a.
IL-1β	≥1·0	6/39 (15%)	12/30 (40%)	2·6 (1·1–6·1)	0·026
IL-1RA	≥78·1	9/52 (17%)	9/17 (53%)	3·1 (1·5–6·4)	0·007
IL-2	≥0·8	5/35 (14%)	13/34 (38%)	2·7 (1·1–6·7)	0·028
IL-4	≥0·5	10/52 (19%)	8/17 (47%)	2·4 (1·2–5·2)	0·035
IL-5	≤1·6	10/51 (20%)	8/18 (44%)	2·3 (1·1–4·8)	0·053
IL-6	≥24·1	9/55 (16%)	9/14 (64%)	3·9 (1·9–8·0)	<0·001
IL-7	≥4·9	10/54 (19%)	7/14 (50%)	2·7 (1·3–5·8)	0·026
IL-9	≥10·4	8/47 (17%)	10/22 (46%)	2·7 (1·2–5·8)	0·018
IL-10	≥3·4	3/33 (9%)	15/36 (42%)	6·9 (1·7–27·8)	0·002
IL-12P70	≥2·0	3/28 (11%)	15/41 (37%)	3·4 (1·1–10·7)	0·017
IL-13	n.a.	n.a.	n.a.	n.a.	n.a.
IL-15	≥5·6	0/26 (0%)	18/43 (42%)	Undefined	<0·001
IL-17A	≥9·9	10/53 (19%)	8/16 (50%)	2·7 (1·3–5·6)	0·021
Growth and colony-stimulating factors (fg/ml)					
FGF-basic	≥5·4	8/42 (19%)	10/27 (37%)	1·9 (0·9–4·3)	0·111
PDGF	≥73·0	3/27 (11%)	15/42 (36%)	3·2 (1·03–10·1)	0·024
VEGF	≥12·1	5/40 (13%)	13/29 (45%)	3·6 (1·4–8·9)	0·004
G-CSF	≥10·5	5/44 (11%)	13/24 (54%)	4·8 (1·9–11·8)	<0·001
GM-CSF	≥12·5	6/41 (15%)	12/28 (43%)	2·9 (1·2–6·9)	0·012

CRP = C-reactive protein; CCL = C-C motif chemokine ligand; CXCL = C-X-C motif chemokine ligand; IFN = interferon; IL = interleukin; FGF = fibroblast growth factor; PDGF = platelet-derived growth factor; VEGF = vascular endothelial growth factor; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; n.a. = the algorithm did not find a threshold respecting the described criteria.

Different explanations can be provided to explain the differences observed between the studies. First, both IL-15 and CCL4 were not included in the analysis of previously mentioned studies [25,26]. Secondly, in our univariate model, the level of IL-6 was significantly different between the patients who died and those who survived, but this was not significant in the multivariate model. This might be due to CRP. As an important risk predictor, CRP might have diminished the role of IL-6, responsible for CRP production, for predicting in-hospital mortality. Indeed, in our previous study we had already found that CRP concentrations were related to death in IE [15], therefore the inclusion of CRP in our risk stratification model was expected. In addition, in our analysis, we used a high-performance Luminex 27-plex assay, which is a novel technique that has higher sensitivity than those used in the other studies. We were therefore able to detect cytokines with a sensitivity of fg/ml. Low undetectable cytokines in their analyses might have been detected and therefore valued in our analysis.

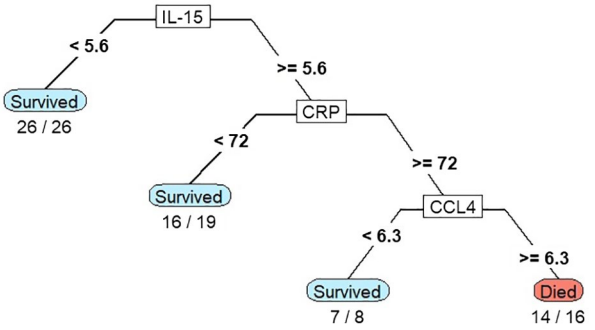

Fig. 1. Classification tree to predict death among infective endocarditis (IE) patients. In each terminal node is shown the prediction and, below, the number of patients with a correct prediction/total number of patients in that node. Unit of interleukin (IL)-15 and C-C motif chemokine ligand (CCL)4 = fg/ml, and of C-reactive protein (CRP) = mg/l.

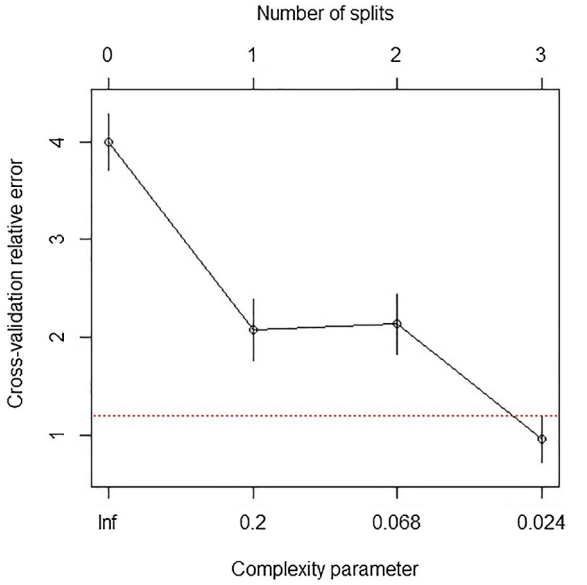

Fig. 2. Cross-validation (69 subgroups) of the model shows that minimum cross-validation relative error occurs with three splits.

IL-15 is a proinflammatory cytokine that is produced by activated monocytes and macrophages. It induces the production of natural killer (NK) cells, and plays an important role in innate immunity [35]. NK cells are able to recognize damaged cells and initiate apoptosis, which is extremely important for clearing a viral infection and for recognition of cancer cells [36]. Endocarditis is a bacterial or fungal infection, and explaining the role of raised IL-15 levels from an innate immunity point of view is difficult. NK cells, however, were also shown to play an important role in the adaptive immunity. NK cells produce a variety of cytokines, chemokines and growth factors that can be both proinflammatory and immunosuppressive, such as IFN-γ, TNF-α, IL-10, GM-CSF, G-CSF, IL-3, CCL2, CCL3, CCL4, CCL5, XCL1 and CXCL8 [36]. The production of all these chemokines leads to attracting other hematopoietic cells such as dendritic cells to the area of inflammation [37]. Reasoning that IL-15 is partly responsible for unleashing this chain reaction may explain why IL-15 is so important in our decision tree. The huge amount of cytokines, chemokines and growth factors that are produced by NK cells due to activation by IL-15 may worsen the inflammation and therefore complicate the IE. As previously described, CCL4 is produced by the NK cells at the site of infection, and our findings show that CCL4 has a prominent place in the decision tree for predicting the outcome of IE. As CCL4 is part of the same chain reaction induced by IL-15, CCL4 seems to play a role in complicating IE. CCL4 is a proinflammatory chemokine and its chemotactic properties are primarily destined for lymphocytes and monocytes [38]. In addition, other inflammatory activities, such as histamine release, have also been registered *in vitro* [38].

IL-15 and CCL4 are both proinflammatory. Adding a possible role of the proinflammatory cytokine IL-6 due to the production of proinflammatory CRP, we conclude that the inflammatory state established by these cytokines and chemokines could complicate the course of IE.

Study limitations and future perspectives

This study has limitations that should be addressed. We conducted the study with a cohort of 69 patients, and analyzed 27 cytokines. Analyzing this amount of cytokines in a small cohort might have influenced the results, and by having a larger sample size other variables may have been shown to be significant predictors of mortality.

Moreover, patients were recruited prospectively and consecutively from a tertiary center in Brazil. We cannot rule out that this may have caused some bias in selecting patients for the study.

Table 4. Risk stratification found by the classification tree.

High-risk group	Died in low-risk group	Died in high-risk group	RR (95% CI)	*P*-value
IL-15 ≥5·6 fg/ml CRP ≥72 mg/dL and CCL4 ≥6·35 fg/ml	4/53 (8%)	14/16 (88%)	11·6 (4·4-30·3)	<0·001

CRP = C-reactive protein; IL = interleukin; CCL = C-C motif chemokine ligand; RR = relative risk; CI = confidence interval.

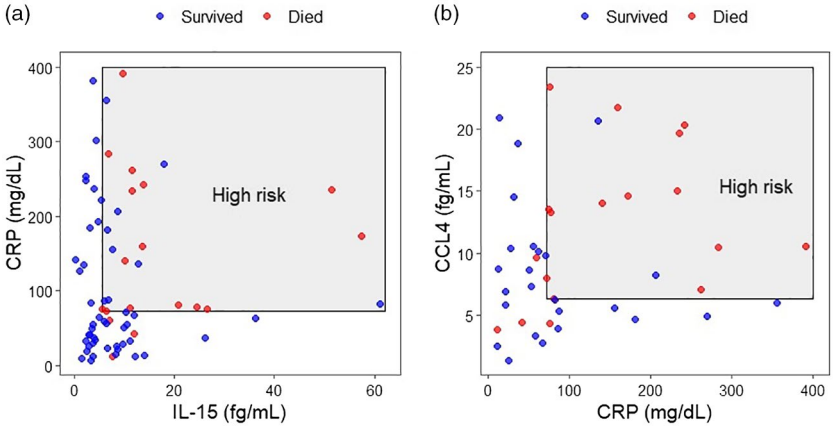

Fig. 3. Illustration of the subgroups found by the CART method. First- and second-decision nodes are illustrated in (a), involving interleukin (IL)-15 and C-reactive protein (CRP), while the second and third decision nodes are illustrated in (b), involving CRP and C-C motif chemokine ligand (CCL)4.

Further research investigating the role of cytokines for early risk stratification in larger IE populations, conducted as a multicenter study, is necessary to create more accurate risk models that are able to be applied in clinical practice when desirable. In addition, at present cytokine measurement is not part of the routine laboratory tests that are performed when suspecting or treating IE. Given this, it will be a challenge to standardize the use of cytokines as supportive evidence for high-risk IE patients in the future.

Conclusions

IL-15 and CCL4 as proinflammatory cytokines were predictors of mortality in IE, adding prognostic value beyond that provided by CRP levels. Assessment of proinflammatory cytokines may offer opportunities to identify patients at risk for complicated IE at an early stage of the disease.

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Disclosure

The authors have no conflicts of interest.

Author contributions

L. R. A. and L. J. S. S. conducted the experiments, M. S. G. and C. B. R. conducted the analysis and data interpretation; A. T. C. coordinated the execution of the experiments with data interpretation; T. J., R. M. P. C., J. T. S., P. H. O. M. P. were responsible for inclusion of the patients, T. C. A. F. and M. C. P. N. contributed to study design, data interpretation and in writing the paper, J. R. H. and N. V. contributed to data interpretation and revised the manuscript.

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