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Alternative Serum Biomarkers of Bacteraemia for Intensive Care Unit Patients

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Abstract **–** The diagnosis of infections in hospital or clinical settings usually involves a series of time-consuming steps, including biological sample collection, culture growth of the organism isolation and subsequent characterization. For this, there are diverse infection biomarkers based on blood analysis, however, these are of limited use in patients presenting confound processes as inflammatory process as occurring at intensive care units. In this preliminary study, the application of serum analysis by FTIR spectroscopy, to predict bacteraemia in 102 critically ill patients in an ICU was evaluated. It was analysed the effect of spectra pre-processing methods and spectral sub-regions on t-distributed stochastic neighbour embedding. By optimizing Support Vector Machine (SVM) models, based on normalised second derivative spectra of a smaller sub-region, it was possible to achieve a good bacteraemia predictive model with a sensitivity and specificity of 76%. Since FTIR spectra of serum is acquired in a simple, economic and rapid mode, the technique presents the potential to be a cost-effective methodology of bacteraemia identification, with special relevance in critically ill patients, where a rapid infection diagnostic will allow to avoid the unnecessary use of antibiotics, which ultimately will ease the load on already fragile patients' metabolism.

Keywords — ***FTIR spectroscopy, Infection, Biomarkers, Intensive Care Unit***

I. Introduction

The diagnosis of infections in a hospital environment, particularly in intensive care units (ICUs), is of critical importance due to the high risk of mortality associated with it [1], [2], with some being nosocomial [3]. However, current methods for identifying infections through culture-based diagnosis are time-consuming and laborious [4]. For this reason, biomarkers based on blood analysis, such as procalcitonin (PCT) [5], [6], C-reactive protein (CRP) [7], [8], interleukin-6 (IL-6) [9], [10] and Cluster of Differentiation 64 (CD64) [11] have been used. However, in the case of critically ill patients, presenting inflammatory conditions, these biomarkers are not specific, as there are systemic signs such as fever, tachycardia, and leucocytosis [12]. This has prompted the search for alternative specific and sensitive biomarkers of infection that can effectively aid in the diagnosis and treatment of infections, especially in critically ill patients.

Fourier Transform Infrared (FTIR) spectroscopic presents diverse characteristics that can enable to achieve that goal, as it enables to acquire the metabolic status of the biological system in a high sensitivity and specificity mode [13]–[15]. Indeed, FTIR spectroscopy has been extensively used in biomedicine applications, especially in the mid-infrared region of the spectra (400cm^{-1} to 4000cm^{-1}), with varied examples of applications including metabolite quantification [16], monitoring of stem cell differentiation [17], transfection events [18], discriminate between B and T-lymphocytes [19], infection processes [20], [21], capturing the human physiological state through serum and plasma analysis [22] [23] [24], as well as for medical diagnosis, prognosis and therapy monitoring [25], [26], [27].

This work aims to evaluate if FTIR spectroscopic analysis of serum would enable to predict bacteraemia in critically ill patients in an ICU environment.

II. Materials and Methods

A. Biological assay

Peripheral blood was collected in a serum tube with no anticoagulant VACUETTE®, using standard blood collection procedures. Samples were maintained at 4ºC until blood centrifugation at 3000 rpm for 10 minutes (Mikro 220T, Hettich, Tuttlingen, Germany). Serum samples were kept at -20ºC up until FTIR spectra acquisition. A total of 102 patients, with COVID-19 and admitted to the ICU of *Hospital São José*, *Centro Hospital Universitário Lisboa Central*, were considered.

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All patients at the time of blood sample collection were submitted to blood microbiological analysis, in which 48 patients presented a bacterial infection (i.e., bacteraemia). All participants provided a signed written informed consent before enrolment in the study approved by the Hospital's Ethics Committee.

B. *FTIR spectra acquisition*

Triplicates of 25 µL of serum diluted at 1/10 in water were transferred to a 96-wells Si plate and then dehydrated for about 2.5 h, in a desiccator under vacuum (ME 2 pump, Vacuubrand, Wertheim, Germany). Spectral data was collected using a FTIR spectrometer (Vertex 70, Bruker, Germany) equipped with an HTS-XT (Bruker, Germany,) accessory. Each spectrum represented 64 coadded scans, with a 2cm^{-1} resolution, and was collected in transmission mode, between 400 and 4000 cm^{-1}. The first well of the 96-wells plate did not contain a sample and the corresponding spectra was acquired and used as background. Medians of triplicate spectra were used.

C. *Spectra pre-processing and processing*

All spectra were submitted to atmospheric correction, using OPUS® software, version 6.5 (Bruker, Germany, Billerica, USA). Second derivative spectra (based on a Savitzky-Golay filter, and a 2rd order polynomial over a 15-point window) and unit vector normalization and spectra processing were conducted by Orange 3 Data Mining Toolbox (Faculty of Computer and Information Science, University of Ljubljana, Slovenia) [28]. Spectra processing included, t-distributed stochastic neighbour embedding (t-SNE), and Support Vector Machine (SVM). Student's *t*-test was performed with Microsoft Excel™ regarding to demographics variables.

III. RESULTS AND DISCUSSION

A total of 102 patients, all hospitalised at an ICU, were considered, from which 48 presented bacteraemia, as based on microbiological analysis. Patients between the two groups (with and without bacteraemia) did not present significant differences concerning gender, age and body mass index ($p>0.1$).

Fig. 1 represents the average spectra between the two groups of patients, after atmospheric and baseline correction, with unit vector normalization (Fig.1A) and the average normalised second derivative spectra (Fig.1B). While normalization minimises the impact of sample quantity under analysis and other variations during experiments, the second derivative resolves superimposed bands, increasing therefore the information retrieved from the spectra. Since derivatives also increase noise, a sub-region of the spectra, with high signal-to-noise ratio was considered, between 600 to 1800 cm^{-1} and between 2800 to 3100 cm^{-1}.

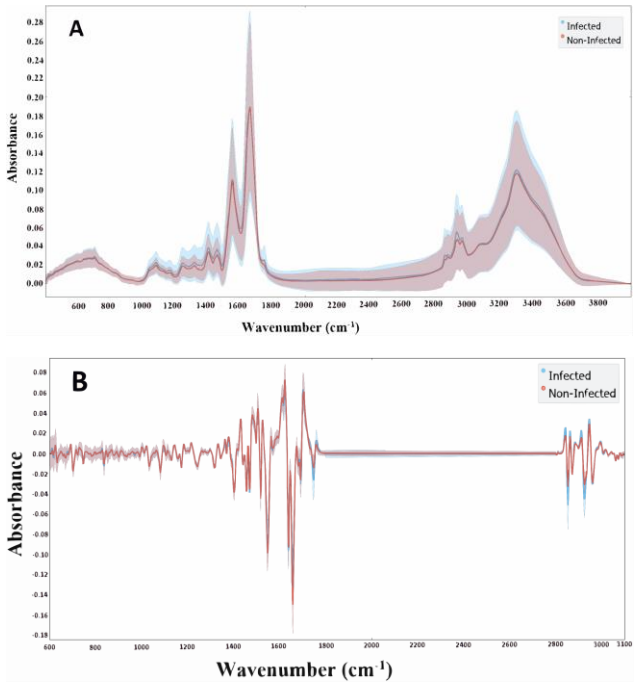

Fig. 1. FTIR pre-processed spectra with atmospheric and baseline correction with unit vector normalization (A) or normalised second derivative spectra (B). In blue, the spectra of the infected group and in red the spectra of non-infected, with shades of the colours representing minimum and maximum values, and solid lines representing their averaged values.

The spectra t-SNE (Fig. 2) points to the advantages of the second derivative spectra, as the scores of the infected group are partially separated from the scores of the non-infected group when based on normalised second derivative spectra (Fig. 2B), and not so when simply using non-derivative spectra (Fig. 2A). To further improve scores separation between the two groups of patients on the t-SNE score-plot, based on the normalised second derivative spectra, a smaller spectral region was selected. In order to achieve this, it was selected the following spectral regions that were statistically different between the two groups of patients at $p<0.001$, as based on an ANOVA: 1375 to 1393 cm^{-1}, 1465 to 1468 cm^{-1}, 1741-1765 cm^{-1}, and between 2837 to 3016 cm^{-1}. It was also selected the following even smaller region, that resulted in a higher statistical significance ($p<0.0001$): 2837 to 3016 cm^{-1}. The t-SNE based on these sub-regions resulted in better scores separation between the two groups of patients (Fig. 2C, D).

Support Vector Machines (SVM) models were developed to predict bacteraemia, based on non-derivative spectra and second derivative spectra based on whole the spectra or sub-regions. For that, 80% of patients' data was used for model training and 20% for independent validation. This process was repeated 100 times, for each spectra pre-processing method and spectral sub-region evaluated. The average model performance obtained from these 100 models are represented in Table 1.

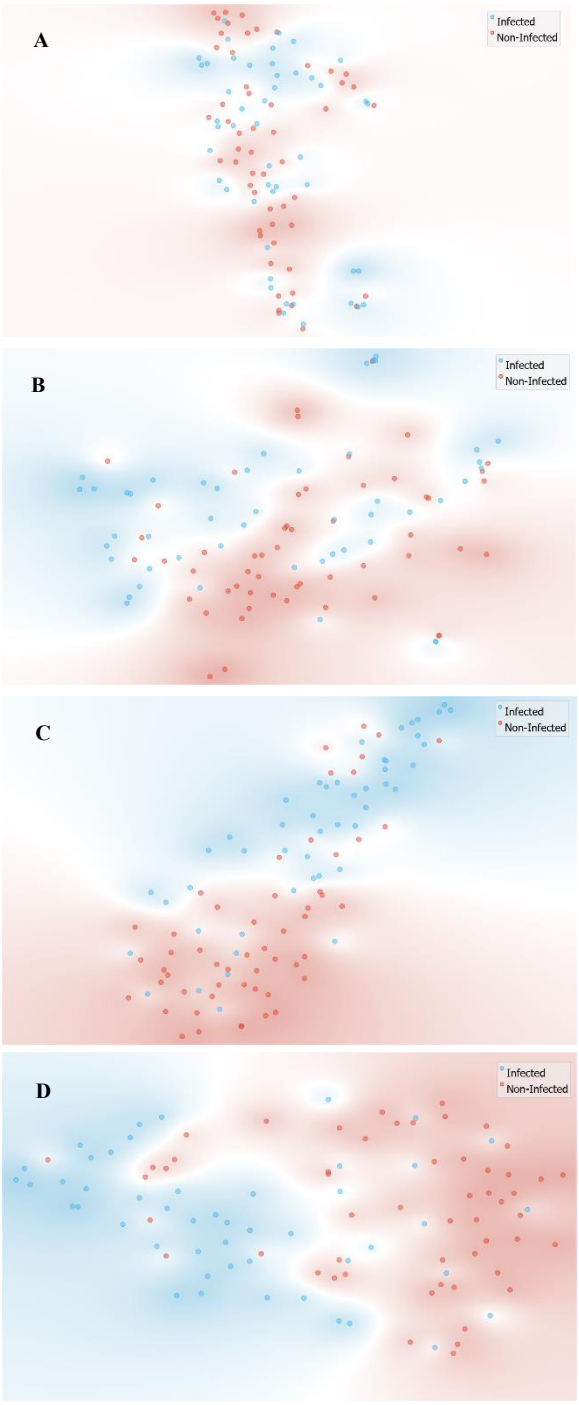

Fig. 2. t-SNE of infected (blue) and non-infected (red) patients, based on serum spectra after baseline correction and normalization (A), normalised second derivative between 600 to 1800 cm^{-1} and between 2800 to 3100 cm^{-1} (B), or between 1375 to 1393 cm^{-1}, 1465 to 1468 cm^{-1}, 1741-1765 cm^{-1}, and between 2837 to 3016 cm^{-1} (C), or between 2837 to 3016 cm^{-1} (D).

According to the previously observed in the t-SNE score-plot, it was only possible to predict bacteraemia, based on SVM models, with normalised second derivative spectra with values for Area Under the Curve (AUC) of a receiver-operating curve higher than 0.72, whereas when based on non-derivative spectra, an AUC lower than 0.5 was obtained.

Table 1. SVM models' performance to predict bacteraemia based on spectra from serum of 102 patients, with baseline correction and normalization (A), normalised second derivative between 600 to 1800 cm^{-1} and between 2800 to 3100 cm^{-1} (B), or between 1375 to 1393 cm^{-1}, 1465 to 1468 cm^{-1}, 1741-1765 cm^{-1}, and between 2837 to 3016 cm^{-1} (C), or between 2837 to 3016 cm^{-1} (D). It is presented the model performance considering 100 models, each based on the random selection of 80% of data for model training and 20% as an independent data set for model validation.

	AUC	Accuracy	Precision	Sensitivity	Specificity
A	0.424	0.513	0.507	0.513	0.498
B	0.725	0.674	0.688	0.674	0.658
C	0.791	0.768	0.767	0.768	0.763
D	0.780	0.750	0.750	0.750	0.748

The best predictive model was achieved based on the spectral sub-regions between 1375 to 1393 cm^{-1}, 1465 to 1468 cm^{-1}, 1741-1765 cm^{-1}, and between 2837 to 3016 cm^{-1}, highlighting the relevance of the regions ≈1750 cm-1 due to νC-O band of the acyl chains, ≈1380 cm-1 associated with δsCH_3 and between 2830 to 3000 cm-1 due to ν and νas of CH_3 and CH_2 groups, all from lipids. This is representative of lipids being a key player in many processes involved in host-pathogen relations, including energy and resource homeostasis, controlling pathogen expression and infection progression [29]. Another highlighted region, ≈1450 cm^{-1} is associated with δsCH_3 and $\delta asCH_3$ from lipids and proteins, also according to the relevance of proteomics in infection diagnosis [30]. The relevance of lipids in the infection process is highlighted by the good prediction model developed on the region between 2837 to 3016 cm^{-1}, which resulted in a decrease of only 1.8% and 1.5% in sensitivity and specificity, respectively, in relation to the best model.

The present work points, therefore, to an alternative mode to detect infections among severe patients, as the ones admitted in an ICU, usually presenting confound factors for the more common serum biomarkers, due to inflammatory process independent of the infection. Therefore, the present method can potentially lead to a rapid detection of infections, leading to an immediate antibiotics-based therapy in severely debilitated patients. Additionally, in the case of a rapid negative result for infection, it would allow to avoid the unnecessary use of antibiotics, that usually are overused in ICU patients, leading to an unnecessary metabolic burden. Furthermore, the technique can be implemented based on simple, rapid and economic mode.

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