Impact Of Serum Metabolome Isolation Process On Models' Prediction Of Critically Ill Patients' Mortality

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1. Introduction

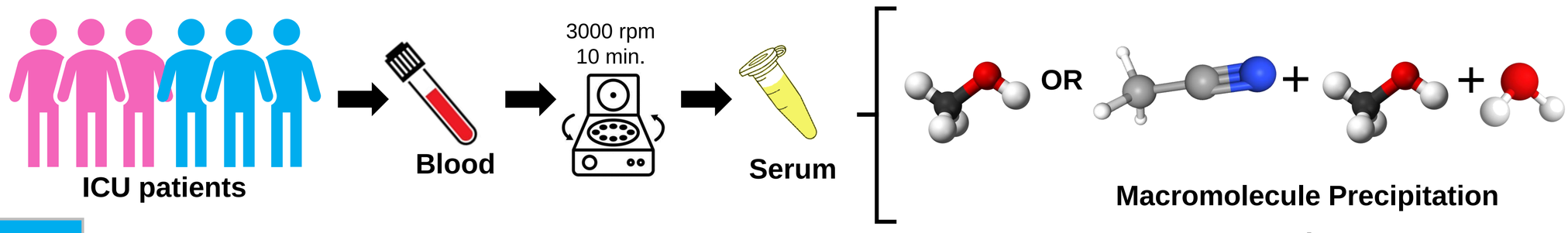
Metabolomics, a downstream approach following genomics, transcriptomics, and proteomics provides a direct reflection of an organism's status. Biofluids metabolomics, such as from serum, has emerged as powerful tool in the discovery of new biomarkers that may be useful during patients' monitoring, especially when they are critically ill (e.g., when admitted to intensive care units) [1]. However, there are numerous challenges, namely the selection of the metabolome isolation process and the platforms to analyze it. FTIR-spectroscopy presents diverse advantages for metabolome analysis, since it may be applied in rapid, economic, and high-throughput mode, while enabling the acquisition of an organism's metabolic status with a high sensitivity and specificity [2,3].

2. Aim

Based on FTIR-spectra and its principal component analysis (PCA), we aimed to evaluate the impact of two different extraction protocols of serum metabolome from critically ill patients, and their reproducibility using multidimensional scaling (MDS). The impact of the two extraction procedures on the performance of predictive models, regarding patients' mortality, was also studied.

3. Methods

Two selected protocols included the precipitation of macromolecules induced by mixtures of methanol with or without acetonitrile, and water. Serum samples from critically ill patients were obtained according to legal and ethics requirements, including project ethics approval by the Hospital Ethics Committee (Centro Hospitalar Universitário Lisboa Central), and patients' informed consent. Replicas of 5 µL of extracted serum metabolome were plated on 384 wells-microplates, and after a rapid dehydration step, spectra were acquired between 400 and 4000 cm^{-1} (**Fig. A**). PCA was used to observe sample distribution/separation regarding the performed protocol (**Fig. B**). The PCA loading score (**Fig. C**) enabled the detection of the main wavenumber regions that separated the samples submitted to the two distinct protocols. The performed MDS enabled to retrieve the relative distances between the samples of each precipitation protocol reveling the relative reproducibility of each method. Finally, resorting to linear discriminant analysis (LDA), a predictive model for patients' mortality was performed to determine which protocol retrieved the best results.



4. Results

Methanol-based extraction yielded molecules rich in functional groups absorbing at wavenumbers 2924 cm^{-1} and 2853 cm^{-1} (asymmetric and symmetric -CH2 vibrations in lipids). Acetonitrile extraction showed a higher proportion of molecules with bands at 1655 cm^{-1} and 1556 cm^{-1} (vibrations from -C-N groups) (**Fig. C**). FTIR spectral data dispersion was identical for both techniques (0.54 for MeOH, 0.51 for Acetonitrile:Methanol), indicating no replicate differences between protocols.

Mortality prediction utilizing PCA-LDA with Methanol-extracted samples achieved 100% accuracy (training) and 95% accuracy (test). Acetonitrile:Methanol-extracted samples yielded a 100% accuracy model for both training and test sets. Overall, Acetonitrile:Methanol extraction demonstrated slightly better results: 100% precision and specificity compared to Methanol (92% and 90%).

5. Conclusions

Distinct molecular profiles were observed between the two extraction protocols. Methanol-extracted samples exhibited an elevated lipidic signature, while samples extracted with a combination of acetonitrile and methanol demonstrated a prominent peptide profile. FTIR spectroscopy offers a valuable means to enhance extraction protocols and construct predictive models for specific phenotypes, such as mortality prediction in critically ill patients. FTIR spectroscopy's advantages encompass its straightforward analysis, high-throughput capability, allowing concurrent analysis small volumed samples.

References

[1] R. Araújo, L.F.N. Bento, T.A.H. Fonseca, C.P. Von Rekowski, B. Ribeiro da Cunha, C.R.C. Calado. (2022). Infection Biomarkers Based on Metabolomics. Metabolites, 2022, 12:92. https://doi.org/10.3390/metabo12020092

[2] B.R. Cunha, L.P. Fonseca, C.R.C. Calado. Simultaneous elucidation of antibiotics mechanism-of-action and potency with high-throughput fourier-transform Infrared spectroscopy and machine-learning. App. Microb. Biot. 2021, 105(3): 1269-1286. doi: 10.1007/s00253-021-11102-7.

[3] R. Araújo, L. Ramalhete, H. Paz, C. Ladeira, CRC Calado. A new method to predict genotoxic effects based on serum molecular profile. Spectrochim. Acta A Mol. Biomol. Spectrosc., 2021 Jul 5;255:119680. doi: 10.1016/j.saa.2021.119680.

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