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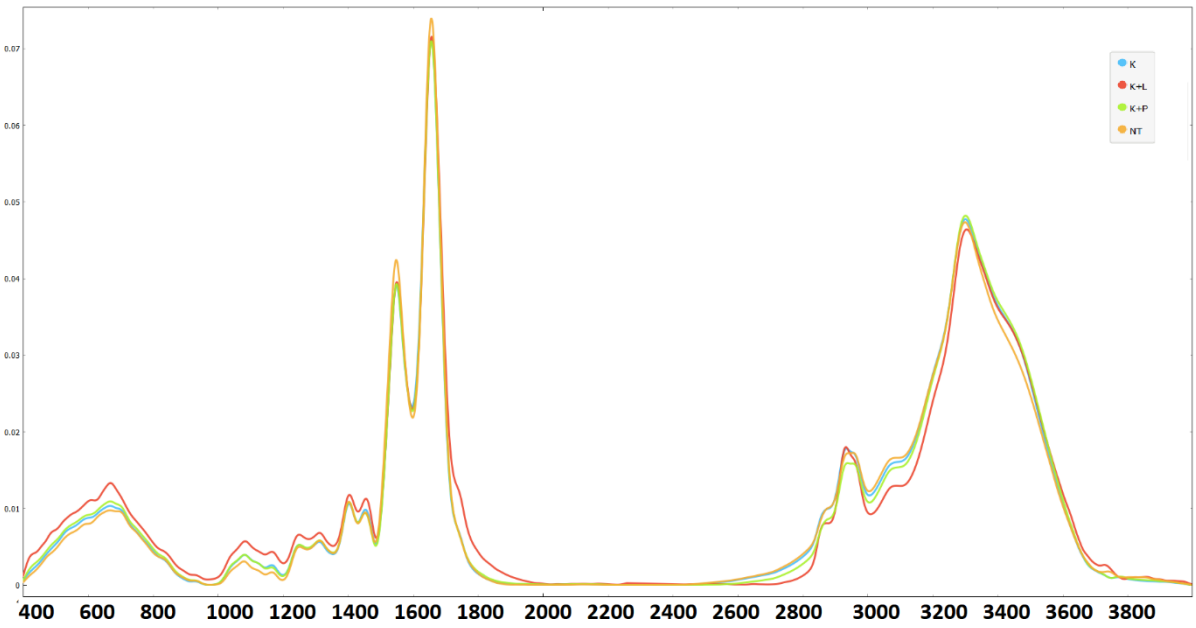

Biomarkers Research for Diagnosis and Prognosis of Rejections of Renal Allograft in Human Transplant by Fourier Transform Infrared Spectroscopy.

Luis Manuel Pires Ramalhete

Thesis to obtain the Master Degree in

Biomedical Engineering

Definitive version

Supervisors:

Cecília Ribeiro da Cruz Calado (ISEL)

December 2019

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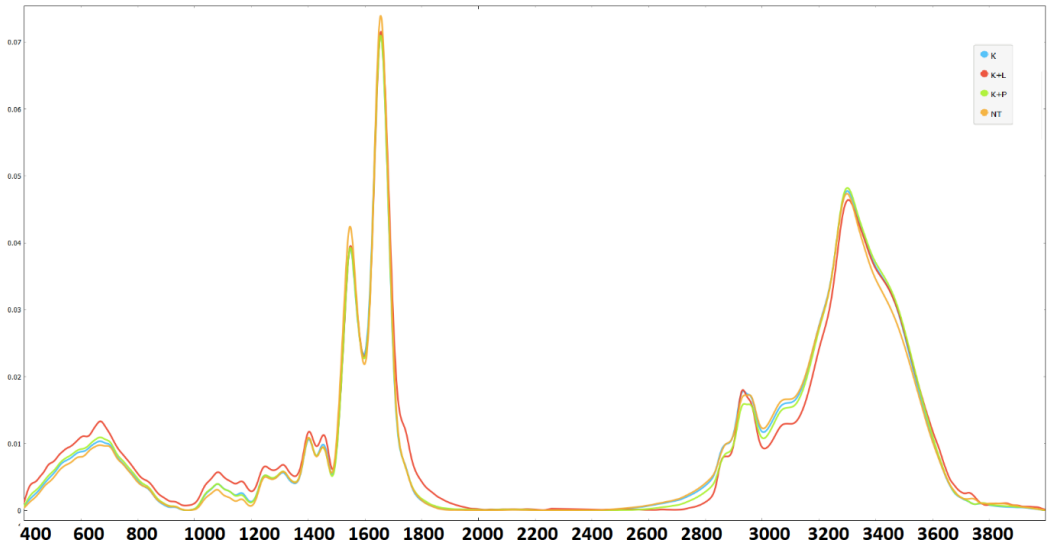

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“The greatest enemy of knowledge is not ignorance,
it is the illusion of knowledge.”

“However difficult life may seem,
there is always something you can do and succeed at.
It matters that you don't just give up.”

Stephen Hawking

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Abstract

Background and main goals: Renal transplantation, when possible, is the treatment of choice for end-stage kidney disease as it enables a much higher quality of life than dialysis. However, one of its major problems is allograft rejection. The present project aims to promote serum biomarkers discovery for diagnosis and prognosis the rejection processes of allograft organs and to evaluate the efficiency of organ rescue therapies, in a rapid, economic but also sensitive and specific mode. It was aimed to develop a new biomarker discovery methodology based on Fourier Transformed Infrared (FTIR) spectroscopy associated to multivariate data analysis and machine learning techniques.

Methods: A total 38 healthy non-transplanted participants, 59 transplanted patients with kidney allograft, from which 12 also received an allograft pancreas or liver, were considered. From the transplanted patients, 29 presented rejection processes. The FTIR spectra was acquired from serum samples of non-transplanted (n=38) and transplanted without (n=213) and with rejection (n=360) processes. It was optimized the dilution degree of the serum samples before spectra acquisition and the spectra pre-processing methods. Diverse spectra unsupervised and supervised processing multivariate data analysis were implemented to search for patterns in data and to develop classification methods to predict the diagnosis, prognosis and the immune mechanisms of rejection and the efficiency of the organ rescue treatment. It was also developed an *in vitro* method based on T-lymphocyte spectra to detect T-cells activation. This assay was based on whole blood samples of 8 healthy non-transplanted volunteers.

Results: It was possible to develop good classification models to predict which patients will develop a rejection process, as for example by Random Forest an AUC of 0.94 was obtained. It was also possible to develop good models to predict the risk of rejection process, as early as 120 days before it was detected in biopsies. For example, by Support Vector Machine an AUC of 0.804, and a sensitivity and specificity of 71.90% and 86.05 % were obtained, respectively. In other models, even before transplanting, it was also possible to predict the risk of rejection, e.g. with a Neural Network model a sensitivity and specificity of 93.33% and 96.55 % were achieved, respectively. In a small cohort of patients (n=20) with rejection processes, and under immunotherapy to minimize the organ lost, it was possible to predict the efficiency of the organ rescue treatment, with e.g. Naïve Bayes or Neural Network models obtaining a AUC of 1.0 with classification accuracy of 0.95 and 0.90 respectively. Considering the serum analysis at the time of biopsy proven cellular (n=12) and humoral (n=42) rejection, it was not possible to develop a good prediction model, probably due to a high mix of immune rejection mechanisms. However, it was possible to identify ratios of spectral peaks based on the 2nd derivative spectra that discriminates humoral from cellular rejection (p<0.05). It was also developed an *in vitro* rapid test (1hr) to detect T-lymphocyte activation from the T-cells spectra that enabled the 100% discrimination, by Hierarchical Cluster Analysis of second derivative spectra, the resting T-cells from activated T-cells.

Conclusions: FTIR spectroscopy of serum coupled with unsupervised and supervised processing multivariate data analysis enabled to develop good predictive models of the rejection diagnosis and prognosis, the risk of rejection before transplantation and the efficiency of the organ rescue treatments. The basis of an *in vitro* method to predict T-lymphocyte activation was also developed, that could enable in the future the rapid prediction of cellular rejection processes. All these new methods, due to simplicity, speed and economic, could increase the monitoring of these type of patients, identify critical patients with an increased risk of rejection processes, and to eventually promoting the adjustment of immunotherapies for organ rescue. These could lead to disrupt modes of management of these type of patients towards a significant increase of quality of life and even of life expectancy, and in a highly economic mode.

Keywords: Organ Rejection, Infrared Spectroscopy, Biomarkers, Machine Learning

Resumo

Enquadramento e objetivos: O transplante renal é o tratamento que proporciona a maior qualidade de vida ao insuficiente renal crónico terminal. No entanto, um dos seus principais problemas é a rejeição do aloenxerto. O presente projeto tem como objetivo promover a descoberta de biomarcadores de diagnóstico e prognóstico de processos de rejeição de aloenxertos renais e avaliar a eficiência de terapias de resgate de órgãos. Pretende-se que o diagnóstico e prognóstico seja efetuado de forma rápida, económica, mas também sensível e específico. Pretendeu-se desenvolver uma nova metodologia de descoberta de biomarcadores baseada na análise por espectroscopia de infravermelho por transformada de Fourier (FTIR) de soro associada à análise multivariada e técnicas de aprendizagem automática.

Métodos: Foram incluídos 38 participantes saudáveis não transplantados, 59 pacientes transplantados renais, dos quais 12 também receberam um aloenxerto de pâncreas ou fígado. Dos pacientes transplantados, 29 apresentaram eventos de rejeição. O espectro de FTIR foi adquirido a partir de amostras de soro de não transplantados (n = 38) e transplantados sem (n = 213) e com (n = 360) rejeição. Antes da aquisição dos espectros foi otimizado o grau de diluição das amostras de soro. Foram implementados diversos métodos de análise multivariada supervisionados e não supervisionados para identificar padrões nos dados e desenvolver métodos de classificação de diagnóstico e prognóstico, de identificação do mecanismo imunológico de rejeição e a eficiência do tratamento de resgate de órgãos. Com base em amostras de sangue total de 8 voluntários saudáveis não transplantado, foi desenvolvido, um método *in vitro* para detetar a ativação de linfócitos T a partir de espectros de células T.

Resultados: Foi possível desenvolver modelos de classificação para prever quais os pacientes que desenvolverão um processo de rejeição, por exemplo por Random Forest, foi obtida uma AUC de 0,94. Também foi possível desenvolver modelos para prever o risco de rejeição, até 120 dias antes de ser detetada em biópsia. Por exemplo, por Máquina de Vectores-Suporte obteve-se uma AUC de 0,804, e uma sensibilidade e especificidade de 71,90% e 86,05%, respetivamente. Foram desenvolvidos modelos de previsão de rejeição com base em amostras obtidas antes do transplante, por exemplo através de um modelo de Redes Neuronais foi alcançado uma sensibilidade e especificidade de 93,33% e 96,55%, respetivamente. Numa pequena coorte de pacientes (n=20) com processos de rejeição e sob imunoterapia para minimizar a perda de órgãos, foi possível prever a eficiência do tratamento de resgate do órgão, por ex. por modelos de Naïve Bayes ou de Redes Neuronais, obteve-se uma AUC de 1,0 com precisão de classificação de 0,95 e 0,90, respetivamente. Considerando a análise sérica no momento da rejeição celular (n=12) ou humoral (n=42) comprovada por biópsia, não foi possível desenvolver um bom modelo de previsão do mecanismo de rejeição com base no espectro. Foi, no entanto, possível identificar bandas espectrais com base na 2ª derivada que discriminam a rejeição humoral da celular (p<0,05). Foi desenvolvido um teste rápido *in vitro* para identificar ativação de linfócitos T, a partir dos espectros de células T, que implicou numa Análise Hierárquica 100% de classificação correta de linfócitos não ativados de ativados.

Conclusões: A espectroscopia de FTIR do soro associada a análise multivariada, permitiu desenvolver modelos preditivos de diagnóstico e prognóstico do processo de rejeição, avaliar o risco de rejeição antes do transplante e a eficiência dos tratamentos de resgate de órgãos. Esta metodologia também permitiu desenvolver um teste *in vitro*, baseado na 2ª derivada do espectro de células T, para prever a ativação dos linfócitos T. Todos esses novos métodos, devido à sua simplicidade, rapidez e economia, poderão identificar pacientes críticos, com maior risco de rejeição, modificando a forma de monitorização desse tipo de paciente, e, eventualmente, promover o ajuste de imunoterapias para o resgate de órgãos, conduzir a novos modos de gestão deste tipo de pacientes que potenciaram um aumento significativo da qualidade de vida.

Palavras-chave: Rejeição de órgãos, Espectroscopia de infravermelho, Biomarcadores, Aprendizagem Automática

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Publications associated with this work:

Book chapter:

B. Cunha, L. Ramalhete, L.P. Fonseca, C.R.C. Calado. Fourier Transformed Mid-Infrared Spectroscopy in Biomedicine. In: Techniques for Medical and Life Scientists: a guide to contemporary methods. e-book, Bentham Science Essential, in processing.

Proceeding:

L. Ramalhete, C.R.C. Calado. Assessing the molecular fingerprint of T lymphocyte activation. ENBENG-2019, IEEE 6th Portuguese Meeting on BioEngineering, 22-23 February 2019, ISEL-Instituto Superior Engenharia de Lisboa, Lisbon, Portugal. DOI: 10.1109/ENBENG.2019.8692471.

Oral communications in conferences:

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L. Ramalhete, C.R.C. Calado. Assessing the molecular fingerprint of T lymphocyte activation. ENBENG-2019, IEEE 6th Portuguese Meeting on BioEngineering, 22-23 February 2019, ISEL-Instituto Superior Engenharia de Lisboa, Lisbon, Portugal. DOI: 10.1109/ENBENG.2019.8692471.

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