From cohort study to the “real life” in patient’s follow-up and personalized medicine using NMR-based clinical metabolomics; results, issues, problems and prospects: the case of neovascular Age-related Macular Degeneration (nAMD)

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Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness among the elderly population in Europe, USA and Australia(1). Clinically, AMD is classified into three forms: early asymptomatic retinal abnormalities (early AMD), the geographic atrophy (GA) and the neovascular or “exudative” form (nAMD). In this last stage, the growth of new blood vessels will progressively cross the retina and finally causes the degeneration of the photoreceptive cells according to a process named choroidal neovascularization (CNV) (Figure 1).

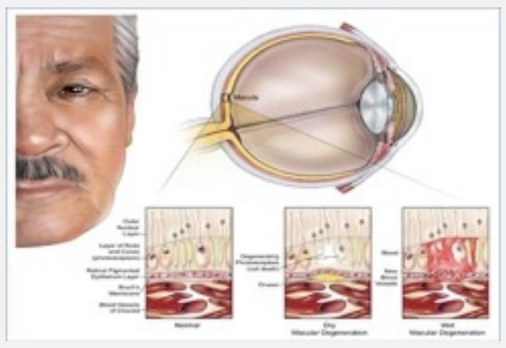

Figure 1. early and exudative macular degeneration

Diagnosis of exudative AMD: ophthalmologic exams

Treatment: intravitreous injection of anti-VEGF

- A continuous follow-up of patient is required
- Treatments stabilizes the vision but are not curative!
- Understanding of Pathogenesis remains incomplete

Major clinical challenges:

- Adapted therapeutic treatments
- Identification of biomarkers as indicator of:
  - Patient stratification
  - Disease progression
  - Treatment responses

Methodology

Our studies was pursuing two main objectives: (1) improve patient’s stratification; and (2) improve patient’s management with a better evaluation of treatment responses and disease progression. For these, we apply a translational metabolomics approach based on proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy on sera collected from AMD patient. All samples were analysed in NMR and multivariate statistics were applied to the processed data to capture changes in the metabolome liked to nAMD evolution.

Case-control study:
- Bleeder AMD patients (n= 49)
- Non-Bleeder AMD patients (n= 23)
- Healthy donors (n= 50)

Plasma samples

^{1}H-NMR untargeted metabolomics:
- Water resonnance suppression
- Apodization
- Baseline correction
- Bucketting
- Normalization

Multivariate analysis:
- PCA
- PLSDA
- OPLSDA

Biomarkers for patients’ stratification

« Real-life » Follow-up:
- 32 “Bleeder” AMD patients followed over 2 year of treatment
- 269 time-points
- Plasma sample
- Clinical parameters:
  - pH
  - Lactate
  - Glucose
  - HDLC, LDLC and Cholesterol
- OCT measurements for AMD status:
  - IRC
  - PED
  - SRF

1H-NMR biomarkers

Evaluation of patients’ status and treatment responses

Figure 4. Overview of the untargeted (left) and targeted (right) metabolomics workflow.

Results from case-study cohort

Lactate and lipoproteins are the most discriminant metabolites

Metabolomics approach does allow differentiation between bleeder and non-bleeder AMD patient after PCA, PLS-DA and O-PLS-DA (Figure 5a-b). Lactate and Lipoproteins emerged as the most discriminant metabolites responsible for this and both NMR and enzymatic dosage of lactate confirmed this finding (Figure 5c-e). These data suggest that lactate could be directly implicated in CNV development as this increased could be related to the bleeding state of the disease(3). NMR analysis of lipoproteins profiles highlights differences between the profile of healthy volunteers and AMD patient (see Figure 5g and 5h). Indeed, our data indicate that VLDL and LDL moieties are increased in patients that develop neovascular AMD suggesting that lipoprotein profiles could be related to the active and non-active phases status of the pathology.

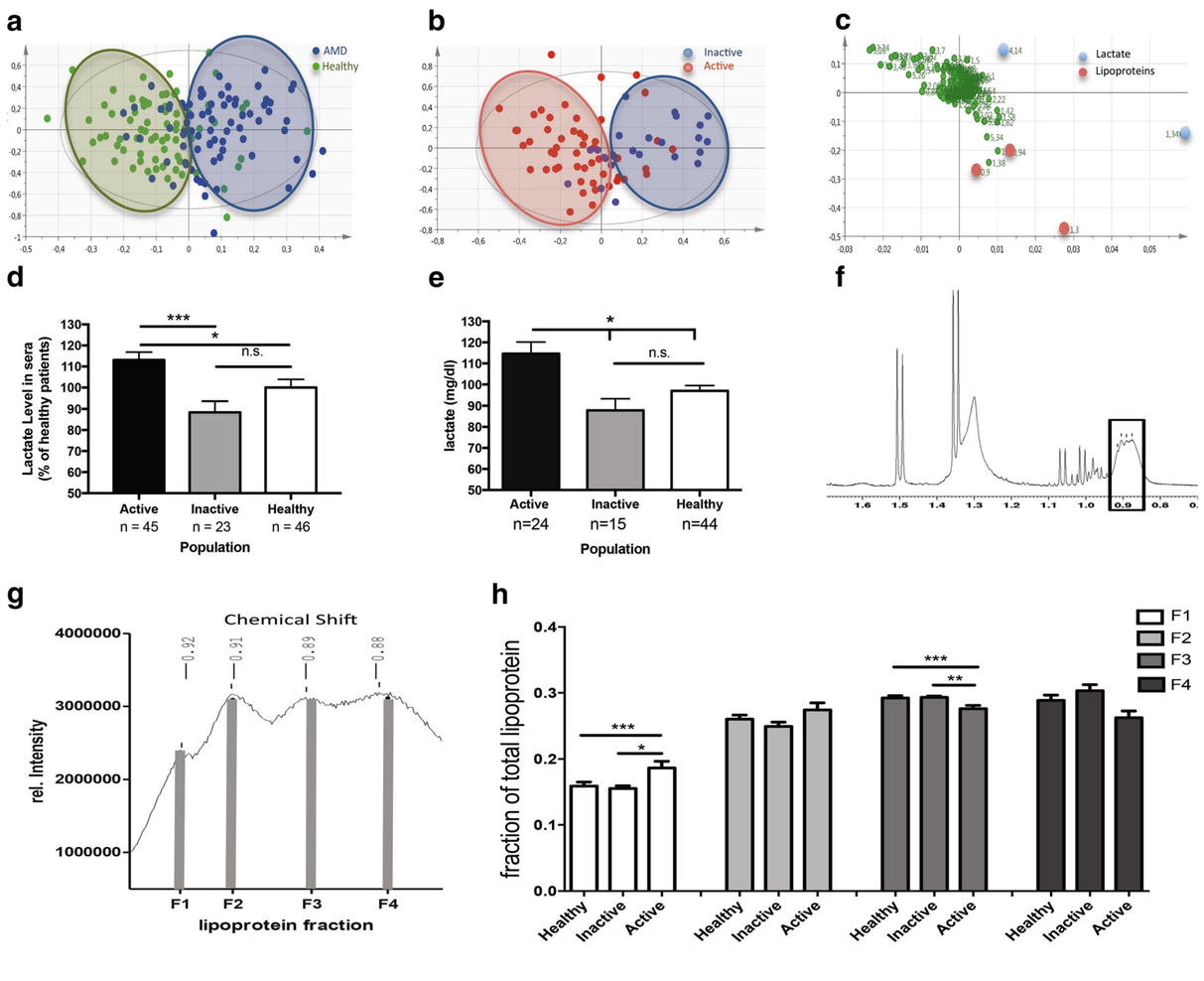

Figure 5. Lactate and lipoproteins are the main increased discriminant metabolite in serum of patients with nAMD. **a** Score plot derived from an OPLS-DA of spectral data (3 components, R2 = 0.527, Q2 = 0.151) collected from patients with nAMD (blue dots, n = 72) and healthy vol- unteers (green dots, n = 50). Each data point represents an individual patient. **b** OPLS-DA score plot (2 components, R2 = 0.468, Q2 = 0.21) of spectral data collected from patients with active (red dots, n = 49) and inactive (blue dots, n = 23) nAMD. **c** Loading plot of spectral data collected from patients with active and inactive nAMD highlighting lactate and lipoproteins as biomarkers of active status. **d** NMR and **e** biochemical dosages of blood lactate in the serum of healthy volunteers and patients with active and inactive forms of AMD. Data are expressed as the percentage of healthy donors. **f** NMR spectrum of human serum highlighting the lipoprotein profiles (from 0.88 to 0.92 ppm). **g** Enlarged view of the lipoprotein NMR spectral zone showing the chemical shift corresponding to the maximum intensity of the signal of the 4 lipoprotein classes. **h** Modification of the lipoprotein profile during CNV development. Fraction 1 is mainly composed of VLDL, while fraction 4 is mainly composed of HDL. *P < 0.05; **P < 0.01; ***P < 0.001. Error bars are SEM .

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“Real-life” follow-up

Results from case-study cohort highlighted lactate and VLDL-rich lipoprotein profile as potential biomarker for active phases of neovascular age-related macular degeneration (nAMD). In order to assess the usefulness of our discoveries, we followed 32 nAMD patients under treatment over a period of two years. At each visits, blood samples and optical coherence tomography (OCT) images of the retina were recorder allowing the quantification of key markers of the disease: (1) intraretinal cystoid fluid (IRC), (2) subretinal fluid (SRF), and (3) pigment epithelium detachment (PED). Through NMR quantification of key metabolites, and evaluation of lipoprotein profiles, we aimed to correlated changes of metabolomes with morphologic changes among the retinas of patients (figure 6).

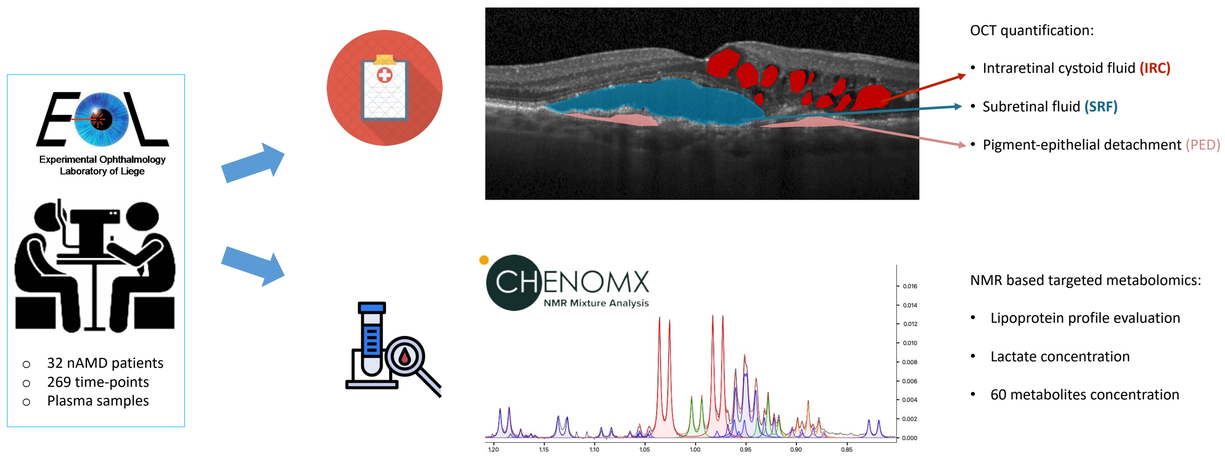

Figure 6. Overview of the targeted metabolomics workflow applied to ”real-lige” nAMD patients’ management.

Unfortunately, our biomarker failed to provide useful information about patients status in real clinical practices. Indeed, neither lactate concentration nor lipoprotein profile were found to be correlated with changes among the retina of nAMD patients (figure 7a). Exploratory PCA analysis were conducted to identify if clusters of individuals that have common disease evolution can be observed and identify metabolites linked to these clusters. But once again, no separation between group of people based on their metabolome could be found (figure 7b-d). Consistently, none of the quantified metabolites were found to be correlated with values coming from OCT images analysis (figure 7e).

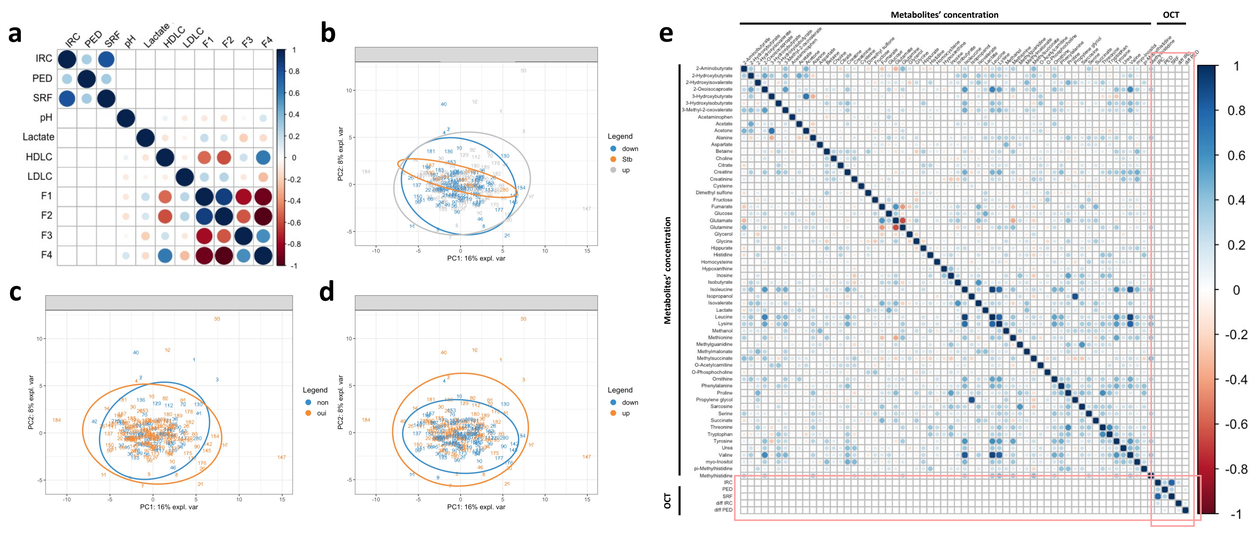

Figure 7. a Pearson’s correlation plot of OCT data, lactate concentration and lipoprotein fraction (F1:VLDL, to F4: HDL) showing possible correlation between continuous variables. **b-d** PCA score plot based on metabolites’ NMR concentrations from nAMD patients’ blood samples. In these plots, individuals are classified regarding the evolution of their **b** IRC, **c** SRF and **d** PED quantified OCT markers. These representation allows the identification of possible clusters of samples. **e** Pearson’s correlation plot of OCT data and quantified metabolites from plasma samples of nAMD patients.

Conclusions

Our first study demonstrates that metabolomics approach can lead to new target and new therapeutical strategies for the treatment and follow-up of AMD patient. Indeed, following lactate level and lipoprotein profiles changes in biofluid samples of AMD patient could help clinician for individual treatment optimization. With these results in hand, we wanted to assess how our marker could be implemented in real-life patients’ management. Unfortunately, when used in real clinical practices, these markers were unable to provide any useful information regarding patient’s status/evolution. Moreover, when tried to use our follow-up cohort to find other metabolites-related markers, none of the analysis provided satisfying results. Even though no consistent results were found with this approach, this work pointed out some important questions regarding how metabolomics research and applications should be used in the context of personalized medicine. Indeed, if the lack of consistency of our previously identified biomarkers can be explained by how far from clinical reality are the case-control studies, forced to see that metabolomics must overcome some challenge to be adapted to the clinic life. By understanding and merging the exigence of both world, metabolomics and in particular NMR-based metabolomics, could play a key role for patient’s monitoring and development of new therapeutical strategies that put the uniqueness of patients in the center of the playground.

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