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#### M064

**Hyperhomocysteinemia is associated with deep vein thrombosis** M. Boudaya<sup>b</sup>, S. Fendri<sup>b</sup>, R. Ben Salah<sup>a</sup>, K. Jamoussi<sup>b</sup>, Z. Bahloul<sup>a</sup>

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#### **Background-aim**

Deep vein thrombosis is a public health problem for which the etiological research is an essential step in the management. Various studies have shown an association between hyperhomocysteinemia and venous thrombosis. We propose during our work to study the relationship between deep vein thrombosis and homocysteine.

# Methods

This was an observational case-control study comparing 47 patients admitted to the internal medicine department aged less than 60 years for the management of deep vein thrombosis confirmed by radiological examination, with negative etiological investigation, with 47 healthy controls. These two groups were matched in age, sex and body mass index. The homocysteine assay was performed by an enzymatic technique.

# Results

The mean age of patients and controls was 40.8  $\pm$  10.5 years with extremes of 18 and 59 years. The two groups consisted of 27 men (57.5%) and 20 women (42.5%) with a sex ratio of 1.35. Homocysteine was significantly (p < 0.001) higher in patients (17.42  $\pm$  8.5 µmol / L) compared to controls (9.41  $\pm$  3.1 µmol / L), with a prevalence of hyperhomocysteinemia (defined by a homocysteine level > 15 µmol / L) of 61.7% in the patients against 4% in the controls. It was found that hyperhomocysteinemie was significantly correlated with the occurrence of deep vein thrombosis with an odds ratio of 3.54 with a 95% confidence interval [1.76 - 16.4] compared to the control group.

## Conclusions

Homocysteine is an intermediate sulfur amino acid in the metabolism of methionine and cysteine. The increase of this metabolite should always be alarming pushing to further investigations and to preventing from thromboembolic complications.

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M065: Biomarker Discovery

Dosage of iohexol in serum and urine by HPLC-UV for direct measurement of glomerular filtration rate: matrix effect, stability and development of the method

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## Background-aim

The determination of glomerular filtration rate (GFR) is essential for the exploration of renal function. The most commonly used marker in clinical practice is creatinine clearance. However, it has limitations in some populations (obese patients) where the precision of GFR measurement is required. Contrast agents such as iohexol could be an excellent alternative as a marker of choice for DFG since he is safe and his clearance could replace that of inulin (reference marker).

## Methods

The objective of our work is the evaluation of the stability, matrix effect and selectivity of the determination of iohexol in serum and urine.

#### Results

The method is linear for both matrices (r2 > 0.99) and the recovery are higher than 98.05%. in practice, no interference was detected. The results of the matrix effect showed a clinically acceptable variation in most concentration levels except for 100 g/ml where there was a slightly significant variation (p < 0.05). Analytes were considered stable under most storage conditions except in urine where stability is significantly decreased after 3 freeze-thaw cycles (p < 0.01) and after freezing to -20 °C for 2 months (p < 0.001).

# Conclusions

According to these results, this method is simple, specific, linear and precise, which allows its application to the direct measurement of the DFG after method validation.

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# M066

COV<sup>2</sup>MS: A tool for simultaneous longitudinal epidemiological monitoring of a variety of pathogens

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## Background-aim

A lesson learned already in the early phase of the COVID-19 pandemic is the need for diagnostic tools that target different biomolecules, using orthogonal experimental setups and fit-for-purpose specification of detection, in addition to the well accepted reverse transcription polymerase chain reaction (RT-PCR).

#### Methods

The Cov-MS effort developed an isotope dilution (based on QconCAT technology) - liquid chromatography mass spectrometry (LC-MS) method that allows accurate, high throughput measurement of SARS-CoV-2 nucleocapsid (NCAP) protein. It uses Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA) technology to enrich and quantify proteotypic peptides of the NCAP protein from trypsin-digested samples from COVID-19 patients. The method is for a bigger part automatable (in terms of the sample preparation, digestion, peptide enrichment and LC-MS measurements).

#### Results

The  $\text{Cov}^2\text{MS}$  assay is compatible with most matrices including nasopharyngeal swabs, saliva and blood plasma, with a sensitivity into the attomole range thanks to the peptide enrichment. The latter also reduces dependency upon LC and allows shortening of LC run time, resulting in the analysis of up to 500 samples per day per MS instrument. There is a strong positive correlation between the SISCAPA antigen

assay and qPCR detection up to a Cycle threshold (Ct) of 30. Importantly, peptide enrichment allowed detection of NCAP protein in a pooled sample containing a single PCR positive patient mixed with 31 PCR negative samples, without loss in sensitivity. Finally, we also demonstrated that it is feasible to rapidly adapt the method for the incorporation of ever emerging variants of concern (VoC), and even other types of respiratory viruses (e.g. Influenza A and B).

# Conclusions

In conclusion, since the Cov<sup>2</sup>MS assay is insensitive to pooling and easily multiplexed, it can provide longitudinal epidemiological monitoring of large numbers of pathogens within a population and can be applied as an early warning system.

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