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Urine Bence Jones proteins as a novel source for De Novo light chain sequencing

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INTRODUCTION

Multiple Myeloma (MM) is a type of cancer of the bone marrow characterized by an abnormal growth of the number of plasma cells, which produce a monoclonal antibody (M-protein). Blood-based mass spectrometric minimal residual disease (MRD) monitoring of clonotypic M-protein peptides requires M-protein sequence information. De Novo protein sequencing is not always possible, for example because serum samples might not be available, or sequences obtained from serum have low coverage due to interferences coming from polyclonal background in sera with low baseline concentration of M-protein which is often the case in light chain (LC) only MM.

Bence Jones proteins secreted in urine might be an ideal source to retrieve the light chain sequence as urine is a cleaner matrix and it is easy to collect.

AIM

The aim of this study is to analyze whether M-protein sequence can be retrieved from urine samples obtained from MM patients.

METHOD

Paired serum/urine samples from 23 MM patients with low M-protein concentrations **(<1 g L^{-1})** were prepped using in-solution protein digestion and ran on an Eclipse mass spectrometer (Thermofisher). De Novo Protein sequence analysis and selection of peptides were performed using several bioinformatics software tools and the paired urine/serum light chain sequences were aligned using an ad-hoc online tool (https://en.vectorbuilder.com/tool/sequence-alignment.html).
Peptides obtained from these sequences were selected for quantitation of M-protein in serum follow-up samples of eight patients using targeted-MS analysis and Skyline software.

RESULTS

Sequence similarity. 19 sequences were obtained out of 23 urine samples. 9 of those sequences show more than 90% similarity with sequences obtained from serum. 4 sequences showed similarity between 80 and 90% and the remaining showed a similarity lower than 80%, the lowest being 62.71% (Table 1). Urine samples allowed a better sequence coverage on 3 out of the 5 patients that did not pass the 95% cut off in serum samples.

Patient	Serum	% Coverage	Urine	% Coverage	% Similarity
1_706	YES	95	YES	94	84
2_625	YES	99	YES	99	88.34
3_791	YES	96	YES	100	91.7
4_731a	YES	91	YES	100	90.05
5_731b	YES	88	YES	100	79.73
6_153	YES	98	YES	100	89.81
7_992	YES	95	YES	85	60
8_421	YES	96	YES	73	79.02
9_809	YES	98	YES	55	77.29
10_127	YES	100	YES	100	100
11_749	YES	97	NO	N/A	N/A
12_846	YES	100	NO	N/A	N/A
13_679	YES	97	YES	93	92.17
14_188	YES	100	YES	91	89.5
15_207	YES	100	NO	N/A	N/A
17_180	YES	89	YES	100	90.14
18_308	YES	96	YES	76	70.51
19_149	YES	100	YES	86	91.89
20_873	YES	91	NO	N/A	N/A
21_181	YES	100	YES	100	98.63
22_114	YES	100	YES	100	99.53
23_176	YES	100	YES	100	99.53
24_634	YES	93	YES	45	64.1

***Table 1**: Percentage of coverage of Light Chain sequence from M-protein obtained from Serum and Urine samples, as well as percentage of similarity.*

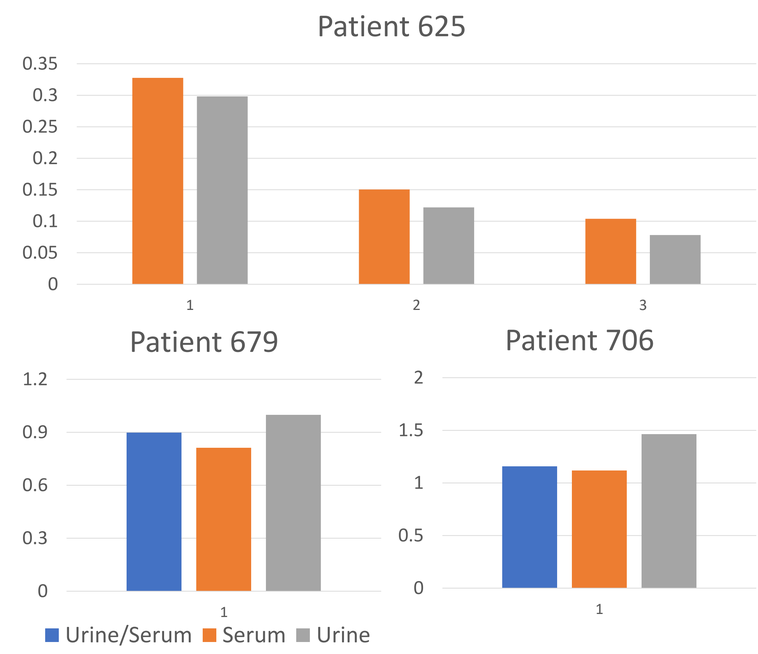

***Figure 1**: Relative M-protein concentration calculated using peptides coming from the sequences found in Urine and Serum samples for three different patients.*

Peptide selection and quantitation. Table 1 shows that higher coverage was found in urine 4 of the patients.

A list of peptides was made for all the 8 patients from which follow-up points were available. This difference in sequencing efficiency can result in clonotyptic peptides found in urine that were not found in their paired serum samples.

Figure 1 shows the M-protein concentration relative to the reference timepoint in 3 of 8 patients. The difference in quantitation between both sequences is lower than 30 % at a concentration below SPEP detection **(<1 g L^{-1}),** Showing that clonotyptic peptides found in **either urine OR serum** can both be used to monitor MRD over time.

CONCLUSIONS

Urine provides a suitable alternative to serum for free light chain M-protein de novo sequencing. Good quality peptides that allow the quantitation of M-protein and MRD-monitoring in serum may be extracted from sequences obtained from urine samples, even in cases where no good candidates are obtained from the serum samples.

REFERENCES

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