A novel mass spectrometry-based assay for the diagnosis and typing of systemic amyloidosis

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INTRODUCTION

The current diagnostic approach for amyloidosis is tedious and relies on several different biochemical techniques. Biomolecular mass spectrometry (MS) can be used in medical diagnosis to identify and quantify protein levels.

OBJECTIVE

To develop an MS-based method that assays in one analysis the major specific systemic amyloidosis proteins, their eventual mutations and several additional proteins that may function as generic indicators of systemic amyloidosis.

PATIENTS AND METHODS

Fat tissue aspirates were processed for liquid chromatography (LC-)MS analysis. A selected reaction monitoring (SRM)-MS assay was developed targeting 13 major systemic amyloidosis proteins (including TTR, Ig-Lambda, Ig-Kappa and SAA) and often occurring mutations on these proteins (11 for TTR, 2 for FGA, 1 for Lys, 1 for ApoA1). Furthermore 20 additional proteins, selected from shotgun proteomics measurements, were included in the assay to evaluate their diagnostic potential for generic systemic amyloidosis. All proteins and mutations were measured using 1 µg of a fat tissue sample during a single MS analysis with a 35 min LC gradient. Heavy isotope-labelled synthetic peptide analogues for all targeted peptides were spiked in the sample to validate detection and enhance quantification. Congo red-stained amyloid in fat smears was graded visually in a semi-quantitative way, ranging from 1+ (<1% of the inspected surface), 2+ (1-10%), 3+ (10-60%) to 4+ (>60%). Fifty-eight samples were used for the training set and 84 samples were used for validation.

RESULTS

Fat tissue aspirates dissolved in Guanidine-HCl were directly processed for LC-MS (trypsin digestion). After untargeted (shotgun) proteomics analysis, 20 proteins were shortlisted as potential systemic amyloidosis signature proteins. Fifty-eight samples for the training set - consisting of control samples and patient samples with varying levels of ATTR, AA, AL-lambda and AL-kappa - were measured with the SRM-assay (Table 1, A). In most cases the amyloidogenic protein was detected; 100% specificity and 75% sensitivity for the whole group. Sensitivity was 100% for unmistakable amyloid (grade 3+ and 4+). Possibly present mutations could also be detected in the same assay.

For validation a set of 84 blinded samples was used (Table 1, B). In most cases the amyloidogenic protein was detected; 100% specificity and 81% sensitivity for the Congo red-positive group. Sensitivity was highest for AA (100%) and ATTR (88%) and lower for AL-kappa (75%) and AL-lambda (63%). Even one Congo red-negative ATTR sample was detected. Correctness of the disease diagnosis increased with increasing amyloid grade: sensitivity was 100% for unmistakable amyloid (grade 3+ and 4+). Amyloid signature proteins could be used to diagnose the presence of amyloid in fat tissue aspirates from systemic amyloidosis patients regardless of type (data not presented). Also the concentration of 'signature' proteins increased with amyloid grade.

Table 1. Amyloid grade of fat tissue and correct typing of amyloid using SRM-MS in **(A)** Training set and **(B)** Validation set

A.

SRM-MS result	Amyloid grade					Controls
Training set	**0**	**1+**	**2+**	**3+**	**4+**	
Amyloid type detected	-	8	10	7	10	0
No amyloid type detected	-	7	5	0	0	11
Sensitivity (%)	-	53	67	100	100	-
Specificity (%)	-	-	-	-	-	100

B.

SRM-MS result	Amyloid grade					Controls
Validation set	**0**	**1+**	**2+**	**3+**	**4+**	
Amyloid type detected	1	8	12	16	16	0
No amyloid type detected	7	8	4	0	0	12
Sensitivity (%)	**13**	**50**	**75**	**100**	**100**	-
Specificity (%)	-	-	-	-	-	**100**

CONCLUSIONS

The SRM-assay we provide here has potential to significantly improve the diagnostic workflow of systemic amyloidosis in the near future. With our assay we can not only reliably and correctly determine the main types of systemic amyloidosis using minimally invasive fat tissue aspirates, but we can also take advantage of the "amyloid signature" to generally detect amyloid deposition in those samples with high accuracy. If aspirates with little amyloid (1+ and 2+) cannot be typed directly, typing can be repeated after laser microdissection of Congo red-positive areas. After applying improvement measures to overcome some limitations, our approach could be implemented in routine diagnosis for systemic amyloidosis. Thereby it would significantly accelerate and facilitate the current workflow of diagnosing and typing of systemic amyloidosis.

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