# Detecting Glycosylation of Monoclonal Light Chain in MGUS Patients by MALDI-TOF MS Based-Assay (MASS-FIX)

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## BACKGROUND

Monoclonal gammopathy of undetermined significance (MGUS) is the most common plasma cell disorder found in approximately 3% of the population over 50 years old [1]. Patients with MGUS are usually asymptomatic and have persistent risk of progression to multiple myeloma or other plasma cell disorders of 1% per year [2]. Since the rate of risk does not decrease over time, lifelong follow-up is required. It is important to identify risk factors to predict groups of MGUS patients with high risk of progression, which can be essential for defining frequency of monitoring, and early diagnosis of multiple myeloma or related disorders.

N-glycosylation of monoclonal light chains has been identified as an important risk factor for progression to primary amyloidosis [3, 4]. A MALDI-TOF MS based-assay with use of isotype-specific nanobody enrichment (MASS-FIX) has been developed and validated to detect and type monoclonal light chains in plasma cell disorders. MASS-FIX is more analytically sensitive, specific, cost-effective, and efficient compared with immunofixation with gel electrophoresis, and it enables easy identification of glycosylated monoclonal immunoglobulins [5].

## OBJECTIVES

Assessing the prevalence of light chain glycosylation at the time of recognition of MGUS.

## METHODS

- Study Cohort: 849 serum samples from unique individuals who lived in the 11 counties of southeastern Minnesota. They had samples collected within 30 days of a previously established MGUS diagnosis at Mayo Clinic, and samples were kept frozen at -80oC until testing.

- Samples were tested for serum protein electrophoresis using agarose gels (Helena Laboratories), immunofixation (Sebia Inc.) and free light chains (FreeLite, Binding Site) were tested by nephelometry on a Siemens BNII.

- MASS-FIX was used to detect glycosylation of the immunoglobin light chains. Serum samples were incubated with agarose beads coupled with antibodies targeting κ or λ light chain constant domains respectively for immuno-enrichment. Beads then washed, reduced, spotted, and analyzed separately on MALDI-TOF MS (Bruker Corporation). The spectra were analyzed using FlexAnalysis software (Bruker Corporation).

- All analyses were conducted using R (version 4.2.1).

## RESULTS

### MASS-FIX workflow

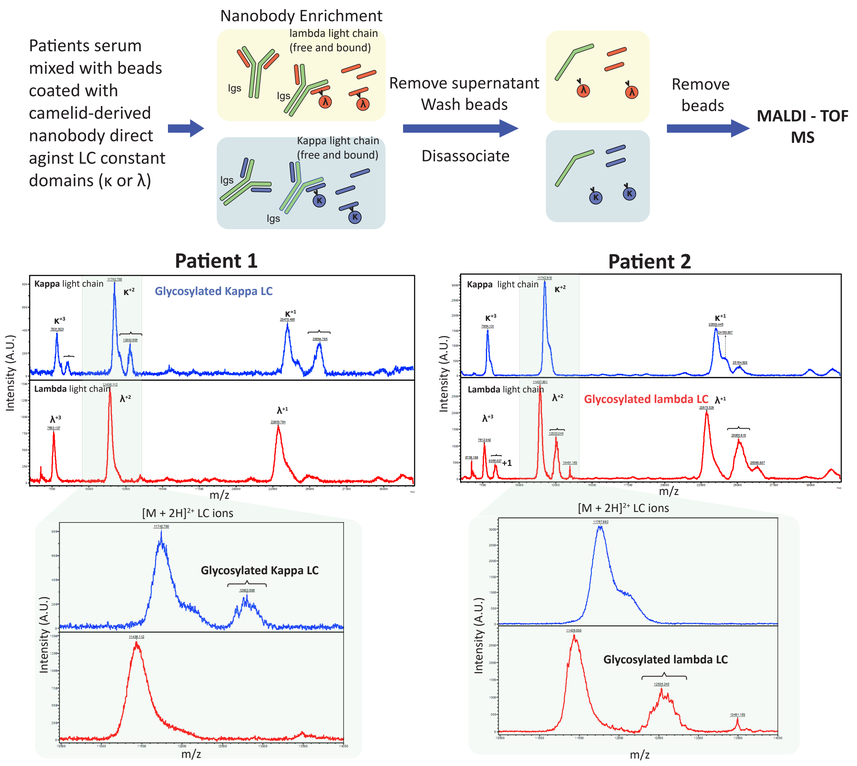


Figure 1. Spectra of patient sample with glycosylated kappa LC (left) and lambda LC (right) detected.

Table 1. Demographics and laboratory characteristics of 849 MGUS patients included in this study.

| | Non-glycosylated (N=804) | Glycosylated (N = 45) | Total (N =849) | P-value |
|---|---|---|---|---|
| **Age** | 72 (24, 96) | 74 (37, 94) | 72 (24, 96) | 0.654[1] |
| **Sex** | | | | 0.241[2] |
| **Female** | 357 (44.4%) | 24 (53.3%) | 381 (44.9%) | |
| **Male** | 447 (55.6%) | 21 (46.7%) | 468 (55.1%) | |
| **Hemoglobin, g/dL** | 13.5 (5.9, 18.0) | 13.2 (9.2, 17.7) | 13.5(5.9, 18.0) | 0.450[1] |
| **Serum creatinine** | 1.1 (0.5, 22.0) | 1.1 (0.7, 3.1) | 1.1 (0.5, 22.0) | 0.550[1] |
| **M-component, g/L** | 1.1 (0.0, 3.0) | 1.2 (0.0, 2.3) | 1.2 (0.0, 3.0) | 0.517[1] |
| **M-spike (SPEP)** | 0.8 (0.2, 4.5) | 0.9 (0.4, 3.8) | 0.8 (0.2, 4.5) | 0.197[1] |
| **BN2 involved LC** | | | | 0.248[2] |
| **K** | 503 (62.5%) | 32 (71.1%) | 535 (63.0%) | |
| **L** | 301 (37.4%) | 13 (28.9%) | 314 (37.0%) | |
| **Sebia involved LC** | | | | 0.211[2] |
| **K** | 497 (61.8%) | 32 (71.1%) | 530 (62.3%) | |
| **L** | 307 (38.2%) | 13 (28.9%) | 321 (37.7%) | |
| **BN2 FLC ratio, n (%)** | | | | 0.100[2] |
| **Normal** | 542 (67.4) | 25 (55.6%) | 567 (66.8%) | |
| **Abnormal** | 262 (32.6%) | 20 (44.4%) | 282 (33.2%) | |
| **Sebia FLC ratio, n (%)** | | | | 0.625[2] |
| **Normal** | 297 (36.9%) | 15 (33.3%) | 312 (36.7%) | |
| **Abnormal** | 507 (63.1%) | 30 (66.7%) | 537 (63.3%) | |

1. Kruskal-Wallis rank sum test; 2. Pearson's Chi-squared test

- Of the total 849 MGUS patients tested, 45 patients (5.3%) were found to have glycosylated light chain.
- Patients with and without glycosylated light chains have similar characteristics

Table 2. MGUS monoclonal protein isotypes from previously published studies.

| | Kyle, R. *et al.* 2006, Olmsted county USA [1] | Han, J. *et al.* 2020. Beijing, China [6] | Eisele, L. *et al.* 2012. German [7] | Iwanda, M. *et al.* 2007. Nagasaki, Japan [8] |
|---|---|---|---|---|
| **Number of people studied** | 21463 | 154597 | 4708 | 52781 |
| **Prevalence of MGUS (%)** | 3.2 (aged 50 years or older) | 1.1 (aged 50 years or older) | 3.5 (aged 45-75 years) | 2.1 (aged 42-98 years) |
| **Number of MGUS patients included** | 694 | 602 | 165 | 1088 |
| **Immunoglobulin isotype (%)** | | | | |
| **IgG** | 68.9 | 65.8 | 58.8 | 73.6 |
| **IgM** | 17.2 | 8.8 | 17.0 | 7.5 |
| **IgA** | 10.8 | 22.4 | 17.0 | 17.6 |
| **IgD** | 0 | 0.3 | 0 | 0.1 |
| **Biclonal** | 3.0 | 2.2 | 4.8 | 1.1 |

- Type of immunoglobulin is a well-established risk factor. MGUS Patients with IgM or IgA monoclonal protein had an increased risk of progression compared to patients who had IgG isotype. Patients with an IgA isotype also had a greater probability for progression to multiple myeloma.

Table 3. Patient without and with glycosylated monoclonal proteins are similar in monoclonal protein isotypes.

| | Non-glycosylated (N=804) | Glycosylated N = 45) | Total (N =849) | P-value |
|---|---|---|---|---|
| **Isotypes** | | | | 0.998[1] |
| **IgG K** | 329 (41.2%) | 23 (51.1%) | 352 (41.8%) | |
| **IgG L** | 214 (26.8%) | 12 (26.7%) | 226 (26.8) | |
| **IgM K** | 86 (10.8%) | 6 (13.3%) | 92 (10.9%) | |
| **IgM L** | 40 (5.0 %) | 0 (0.0%) | 40 (4.7%) | |
| **IgA K** | 59 (7.4%) | 2 (4.4%) | 61 (7.2%) | |
| **IgA L** | 38 (4.7%) | 1 (2.2%) | 39 (4.6%) | |
| **Others (biclonal or triclonal)** | 31 (4.0%) | 1 (2.2%) | 32 (3.5%) | |
| **Isotype heavy chain** | | | | 0.220[1] |
| **IgG or Biclonal IgG** | 571 (71.6%) | 36 (80.0%) | 607 (72.0%) | |
| **IgA, IgM, or IgA and IgM** | 227 (28.4%) | 9 (20.0%) | 236 (28.0%) | |

1. Kruskal-Wallis rank sum test; 2. Pearson's Chi-squared test

- Consistent with previous studies [1, 6-8] , the most common isotype of monoclonal protein was IgG in 68% of 849 MGUS patients. IgM in 15.8%, IgA in 12.1%, and biclonal in 4%.

## CONCLUSION

- The prevalence of light chain glycosylation at the initial diagnosis of MGUS is about 5%.

- Glycosylation further characterization can provide valuable information on disease development, and follow-up information from patients over time may add value in stratifying patients into groups that would be at higher risk of progression to a malignancy at early stages of MGUS.

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