Use of Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) Free Light Chain Assessment for the Diagnosis and Monitoring of Systemic Immunoglobulin Light Chain (AL) Amyloidosis

Joshua Bomsztyk[1*], Sriram Ravichandran, MBBS, MRCP, FRCPath[2*], Hannah Victoria Giles, FRCPath, MRCP[3*], Oscar Berlanga, PhD[4*], Stephen Harding, PhD[5], Jahanzaib Khwaja[6*], Shameem Mahmood, MD[7,8*], Brendan Wisniowski, MD[7,9*], Oliver C Cohen, MBBS, BSc, FRCPath[10*], Darren Foard[11*], Janet Gilbertson[12*], Ana Martinez De Azcona Naharro[12*], Lucia Venneri[12*], Carol Whelan[10*], Marianna Fontana[12*], Philip N Hawkins, MD PhD[13*], Julian D Gillmore[13*], Helen J Lachmann[13*], Guy Pratt, MD FRCP FRCPath[14,15*] and Ashutosh D. Wechalekar[9,16]

[1]Royal Free Hospital, National Amyloid Centre, London, United Kingdom; [2]National Amyloidosis Centre, Royal Free Hospital, London, West Bengal, United Kingdom; [3]University Hospitals Birmingham NHS Foundation Trust and the University of Birmingham, Birmingham, United Kingdom; [4]The Binding Site Group Ltd, Birmingham, United Kingdom; [5]The Binding Site Inc., Birmingham, United Kingdom; [6]Department of Haematology, University College London Hospital, London, United Kingdom; [7]Department of Haematology, University College London Hospitals NHS Foundation Trust, London, United Kingdom; [8]Haematology, National Amyloidosis Centre, London, United Kingdom; [9]National Amyloidosis Centre, London, United Kingdom; [10]National Amyloidosis Centre, Royal Free Hospital, London, United Kingdom; [11]National Amyloidosis Centre, National Amyloidosis Centre, Royal Free Hospital, London, United Kingdom; [12]National Amyloid Centre, London, United Kingdom; [13]Royal Free Hospital, National Amyloidosis Centre, London, United Kingdom; [14]University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; [15]School of Cancer Sciences, University of Birmingham, Birmingham, United Kingdom; [16]University College London Hospitals NHS Foundation Trust, London, United Kingdom

Introduction

AL amyloidosis is characterised by fibrillar deposition of amyloidogenic light chains causing progressive organ failure. Measurement of serum free light chains

(sFLC) transformed the diagnosis and management of amyloidosis; but limitations include the inability to detect the monoclonal FLC component in some patients and difficulties in clonal assessment at low serum levels or in renal impairment. MALDI-TOF MS to measure sFLC shows promise to overcome many of these limitations. We report the first large series of prospectively followed patients with AL amyloidosis assessed using MALDI-TOF MS to detect sFLC and report the concordance with routine methods and the impact on survival outcomes and organ responses.

Methods

Serum samples from patients in a prospective observational study of newly diagnosed AL amyloidosis (ALCHEMY) were analysed at diagnosis and at 6 post-diagnosis. All patients underwent serial protocolised amyloidosis assessments. Diluted serum samples were incubated with polyclonal antisera specific for κ and λ FLC conjugated to magnetic microparticles. Captured FLC were eluted and reduced, spotted onto target plates and analysed by MALDI-TOF MS. The presence or absence of monoclonal FLC was denoted as FLC-MS positive and FLC-MS negative. Landmark analysis was undertaken at 6 months.

Results

487 patients are included. The median age was 67 years (range 36 – 88) and 378 (77.9%) had ECOG performance status <2. 290 (59%) had cardiac involvement and 256 (52.4%) had renal involvement. Cardiac disease stage (Mayo European modification) was stage I – 99 (20.3%), II – 178 (36.5%), IIIa – 167 (34.2%) and IIIb – 39 (8%). 256 (52.4%) patients had ≥2 organs involved. The involved free light chain (iFLC) was κ in 90 (18.5%), λ in 395 (81.1%) and unidentified in 2 (0.41%) by Freelite assay. Median iFLC at presentation was 197mg/l (range 11.6 – 15,900mg/l) and difference between involved and uninvolved light chain (dFLC) was 177.7mg/l (range 0 – 15,898mg/l).

Based on tissue fibril typing by immunohistochemistry or proteomics 53 (10.9%) had κ AL-type and 292 (60.0%) had λ AL-type. There was 100% concordance in the light chain isotype identified by FLC-MS. Comparing FLC-MS vs. sFLC measurement by Freelite assay, 88 (97.8%) patients with a κ iFLC had a concordant κ clone by FLC-MS and 393 (99.5%) patients with a λ iFLC had a concordant λ clone by FLC-MS. 4 (0.8%) patients did not have a FLC clone detected by FLC-MS. Of patients presenting with a normal sFLC ratio, FLC-MS identified a clone in 113/114 (99.1%) patients. Using FLC-MS 26 patients (5.3%) were biclonal (κ and λ).

After treatment at 6 months, the haematological responses by standard ISA criteria were: complete response (CR) 159 (32.6%), very good partial response (VGPR) 167 (34.3%), partial (PR)/no response (NR) 161 (33.1%). 82 (16.8%) patients were MS-FLC negative of which 42 (26.4%), 34 (20.4%) and 6 (3.7%) patients were in CR, VGPR and PR/NR respectively (Table 1). 50/199 (25.1%) of patients with dFLC <10mg/l and 45/154 (29.2%) patients with iFLC<20mg/l were FLC-MS negative.

At 6 months, there was significantly better overall survival (OS) for patients who were FLC-MS negative vs. positive (median not reached vs. 63 months ($p<0.001$)). For those achieving a CR/ VGPR, there was significantly better OS for patients who were FLC-MS negative vs. positive, median OS not reached vs. 80 months ($p = 0.038$) and median OS 85 vs. 60 months ($p = 0.029$), for patients in CR and VGPR respectively (FIg.1)

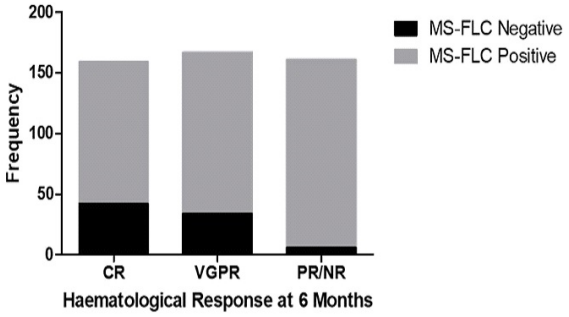
261 (90%) patients with cardiac involvement and 302 (86.6%) with renal involvement were assessable for response at 6 months. In the FLC-MS (negative vs. positive patients), cardiac response was seen in 15/42 (35.7%) vs. 56/248 (22.6%) ($p=0.0867$) and renal response in 15/63 (23.8%) vs. 52/286 (18.2%) ($p=0.453$).

Conclusion

Detection of clonal FLC by MS shows 100% concordance with amyloid fibril typing from tissue biopsies and can identify a FLC clone in patients presenting with a normal FLC ratio and in the majority of patients in conventional CR post treatment. After treatment, patients with no detectable residual monoclonal FLC by FLC-MS had significantly better OS compared to patients with a conventional CR/VGPR but remaining FLC-MS positive. Detailed organ response data will be presented. FLC assessment by MALDI-TOF MS represents a crucial advance in the diagnostic armamentarium for the identification and monitoring of monoclonal

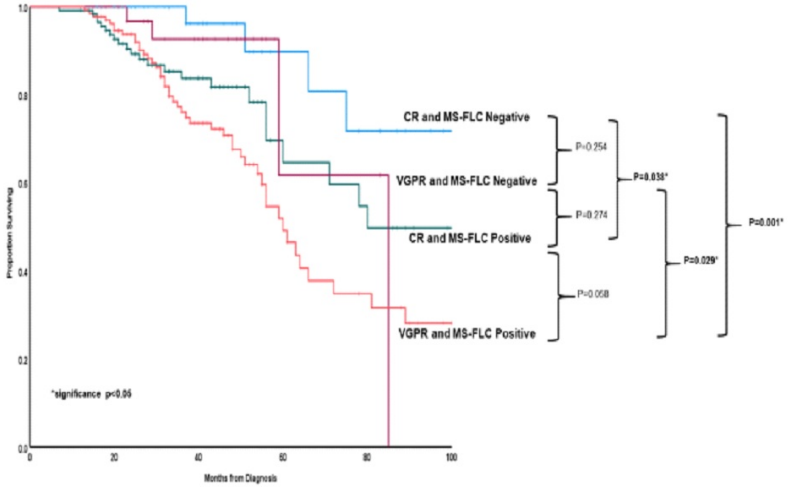
FLC in patients with AL amyloidosis.

Table 1. Frequency of FLC-MS negative at 6-month assessment based on haematological response
Complete response CR; Very good partial response VGPR; partial response PR; no response NR; Free Light Chain by Mass Spectrometry (MALDI-TOF MS) FLC-MS



	MS-FLC Negative	MS-FLC Positive
CR (n = 159) (%)	42 (26.4%)	117 (73.6%)
VGPR (n = 167) (%)	34 (20.4%)	133 (79.6%)
PR/NR (n = 161) (%)	6 (3.7%)	155 (96.3%)

Figure 1. Overall survival from diagnosis at 6-month landmark analysis based on haematological response and presence/absence of MS-FLC
Complete response CR; Very good partial response VGPR; Free Light Chain by Mass Spectrometry (MALDI-TOF MS) MS-FLC



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First Presenter

Presenter
Corresponding Presenter
Joshua Bomsztyk
National Amyloid Centre
Rowland Hill Street London
Royal Free Hospital
London, NW3 2PF
United Kingdom
Email: Joshuabomsztyk@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/27/2022 by ***Joshua Bomsztyk, MBChB, MRCP, DipRCPath***

Second Author

Sriram Ravichandran, MBBS, MRCP, FRCPath
National Amyloidosis Centre, Royal Free Hospital
Rowland Hill St
14, MAR(EW), Rajarhat, New Town
London, West Bengal NW3 2PF
United Kingdom
Email: sriram.ravichandran@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Sriram Ravichandran, MBBS, MRCP, FRCPath***

Third Author

Hannah Victoria Giles, FRCPath, MRCP
University Hospitals Birmingham NHS Foundation Trust and the University of Birmingham
Birmingham, B15 2GW
United Kingdom
Email: hannah.giles3@nhs.net -- Will not be published

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Signed on 07/27/2022 by ***Hannah Victoria Giles, FRCPath, MRCP***

Fourth Author

Oscar Berlanga, PhD
The Binding Site Group Ltd
8 Calthorpe Road
Birmingham, B15 1QT
United Kingdom
Email: oscar.berlanga@bindingsite.co.uk -- Will not be published

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Fifth Author

Stephen Harding, PhD
The Binding Site Inc.
8 Calthorpe Road
Edgbaston
Birmingham, B15 1QT
United Kingdom
Email: stephen.harding@bindingsite.co.uk -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? Yes

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Signed on 07/28/2022 by ***Stephen Harding, PhD***

Sixth Author

Jahanzaib Khwaja
University College London Hospital
Department of Haematology
London,
United Kingdom
Email: j.khwaja@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/27/2022 by ***Jahanzaib Khwaja***

Seventh Author

Shameem Mahmood, MD
University College London Hospitals NHS Foundation Trust
University College Hospital, 235 Euston Road
Department of Haematology
London, NW1 2BU
United Kingdom

National Amyloidosis Centre
Rowland Hill Road
Haematology
London, NW32PF
United Kingdom
Email: shameem.mahmood@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Shameem Mahmood, MD***

Eighth Author

Brendan Wisniowski, MD
University College London Hospitals NHS Foundation Trust
University College Hospital, 235 Euston Road
Department of Haematology
London, NW1 2BU
United Kingdom
National Amyloidosis Centre
Rowland Hill Street
London, NW3 2PF
United Kingdom
Email: brendan.wisniowski@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Brendan Wisniowski, MD***

Ninth Author

Oliver C Cohen, MBBS, BSc, FRCPath
National Amyloidosis Centre, Royal Free Hospital
Rowland Hill St
London, NW3 2PF
United Kingdom
Phone Number: 07813212501
Email: o.cohen@nhs.net -- Will not be published
Alternate Email: o.cohen@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Oliver C Cohen, MBBS, BSc, FRCPath***

Tenth Author

Darren Foard
National Amyloidosis Centre, Royal Free Hospital
Rowland Hill St

National Amyloidosis Centre
London, NW3 2PF
United Kingdom
Email: d.foard@ucl.ac.uk -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Darren Foard***

Eleventh Author

Janet Gilbertson
National Amyloid Centre
Rowland Hill Street London
London, NW3 2PF
United Kingdom
Email: janet.gilbertson@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Janet Gilbertson***

Twelfth Author

Ana Martinez De Azcona Naharro
National Amyloid Centre
Rowland Hill Street London
London, NW3 2PF
United Kingdom
Email: anamartinez.naharro@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Ana Martinez De Azcona Naharro***

Thirteenth Author

Lucia Venneri
National Amyloid Centre
Rowland Hill Street London
London, NW3 2PF
United Kingdom
Email: lucia.venneri@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Lucia Venneri***

Fourteenth Author

Carol Whelan
National Amyloidosis Centre, Royal Free Hospital
Rowland Hill St
London, NW3 2PF
United Kingdom
Email: c.whelan@ucl.ac.uk -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? Yes

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Signed on 07/28/2022 by ***Carol Whelan***

Fifteenth Author

Marianna Fontana
National Amyloid Centre
Rowland Hill Street London
London, NW3 2PF
United Kingdom
Email: marianna.fontana@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Marianna Fontana***

Sixteenth Author

Philip N Hawkins, MD PhD
National Amyloidosis Centre
Rowland Hill St
Royal Free Hospital
London, NW3 2PF
United Kingdom
Email: p.hawkins@ucl.ac.uk -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Philip N Hawkins, MD PhD***

Seventeenth Author

Julian D Gillmore
National Amyloidosis Centre
Rowland Hill St
Royal Free Hospital
London, NW3 2PF
United Kingdom
Email: julian.gillmore@nhs.net -- Will not be published

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Signed on 07/28/2022 by ***Julian D Gillmore***

Eighteenth Author

Helen J Lachmann
National Amyloidosis Centre
Rowland Hill St
Royal Free Hospital
London, NW3 2PF
United Kingdom
Email: h.lachmann@nhs.net -- Will not be published

In the past 24 months, have you had any financial relationships with an ineligible company as defined above? No
Signed on 07/28/2022 by ***Helen J Lachmann***

Nineteenth Author

Guy Pratt, MD FRCP FRCPath
University Hospitals Birmingham NHS Foundation Trust
Birmingham,
United Kingdom
University of Birmingham
Vincent Drive

Edgbaston
School of Cancer Sciences
Birmingham,
United Kingdom
Email: guy.pratt@uhb.nhs.uk -- Will not be published

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Twentieth Author

Ashutosh D. Wechalekar
National Amyloidosis Centre
Royal Free Hospital
London, NW3 2PF
United Kingdom
Phone Number: 44 (791) 9078729
University College London Hospitals NHS Foundation Trust
London,
United Kingdom
Email: a.wechalekar@ucl.ac.uk -- Will not be published

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