

# QUANTIFICATION OF IGA $\kappa$ / IGA $\lambda$ IN MONOCLONAL GAMMOPATHIES

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## Introduction

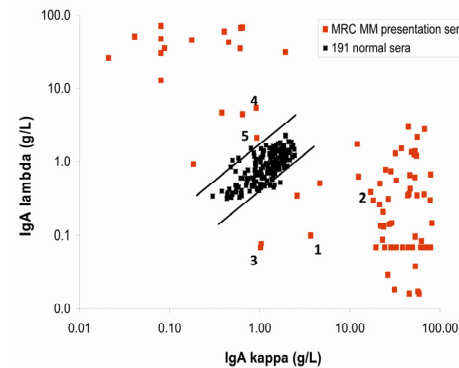
Antibodies recognising specific epitopes on IgA $\kappa$  and IgA $\lambda$  have been used to develop nephelometric assays. Here we describe their use in sera from multiple myeloma (MM) patients at presentation and throughout treatment.

## Materials and Methods

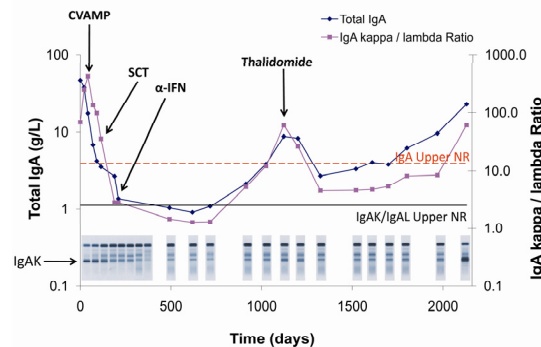
IgA $\kappa$ /IgA $\lambda$  ratios were measured in 191 blood donor sera to generate a normal range. Archived MM sera collected in Medical Research Council myeloma trials were analysed. 83 IgA (65 IgA $\kappa$ : 18 IgA $\lambda$ ) patient sera were analysed at presentation, with serial sample analysis being completed on 32 patients (20 IgA $\kappa$ : 12 IgA $\lambda$ ). Kaplan Meier survival curves were generated using SPSS v14.0.

## Results and Discussion

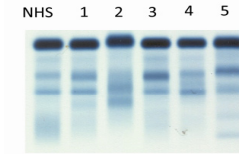
For 191 normal sera, median values were: IgA $\kappa$  1,270 mg/L (range 440-2,360); IgA $\lambda$  870 mg/L (range 340-1,850); IgA $\kappa$ /IgA $\lambda$  ratio 1.47 (range 0.58-2.52). The summation of IgA $\kappa$  and IgA $\lambda$  correlated well with total IgA ( $r^2=0.95$ ) for all samples tested. In all presentation sera and all patient samples during monitoring, the appropriate immunoglobulin levels and/or the IgA $\kappa$ /IgA $\lambda$  ratios were abnormal. In contrast 23/83 presentation sera were not accurately quantifiable by serum protein electrophoresis (SPE) (for 5 examples see fig 2). In 32 patients followed through their disease, the IgA $\kappa$ /IgA $\lambda$  ratios were in accordance with overall clinical assessments and at least matched the sensitivity of IFE for detection of residual disease. In 10/32 patients accurate quantification of the paraprotein by SPE was impossible (for example see fig 3). The mean IgA $\kappa$ /IgA $\lambda$  or IgA $\lambda$ /IgA $\kappa$  ratio for the 83 presentation sera was 449 (range 4.96 – 3675). Patients with a value >449 (n=20) had a significantly worse outcome (fig 4) compared to those with a lower ratio (n=52, 11 incomplete data sets) with a median survival of 23 v 34 months (p=0.05).



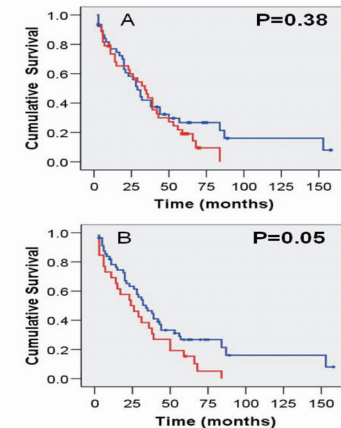
**Figure 1:** Plot of IgA $\kappa$  v IgA $\lambda$  for 191 blood donor sera and 83 presentation sera from MRC multiple myeloma trials. The parallel lines indicate the 95%ile range for IgA $\kappa$  /IgA $\lambda$  ratios.



**Figure 3:** Serial analysis of an IgA $\kappa$  patient's sera throughout their disease. The monoclonal protein migrated into the  $\beta$  region of the SPE gel making accurate quantification by SPE densitometry difficult. The patient responded well to treatment, total IgA and the IgA $\kappa$ /IgA $\lambda$  ratio fell within the normal range after autologous stem cell transplant. Abnormal IgA $\kappa$  /IgA $\lambda$  ratios indicated relapse 209 days before total IgA rose above the upper limit of the normal range. CVAMP= cyclophosphamide, vincristine, adriamycin, melphalan and prednisone, SCT= stem cell transplant,  $\alpha$ IFN= alpha-interferon, NR= normal range.



**Figure 2:** SPE of 5 samples which were not quantifiable by SPE densitometry but were abnormal for IgA $\kappa$  or IgA $\lambda$  and the IgA $\kappa$ /IgA $\lambda$  ratio (numbers correspond to those in figure 1). NHS= normal human sera.



**Figure 4:** Kaplan Meier survival curve for 83 IgA MM patients comparing A) IgA immunoglobulin measurements above (red line) and below (blue line) the mean value (38.1 g/L). B) heavy/light chain ratios (IgA $\kappa$ /IgA $\lambda$  or IgA $\lambda$ /IgA $\kappa$ ) above (red line) and below (blue line) the mean value (449). Patients with heavy / light chain ratios above the mean value had a significantly poorer outcome.

## Conclusion

Using nephelometric assays to quantify IgA $\kappa$  and IgA $\lambda$  it was possible to monitor response to therapy even in patients with difficult to interpret SPE results. In some instances the ratios provided a more sensitive indication of residual disease than IFE, and importantly, the results were quantitative rather than qualitative. Finally, preliminary data indicates that extreme ratios are associated with shorter overall survival.