

Separate nephelometric immunoassays for IgG κ and IgG λ for the assessment of patients with multiple myeloma (MM)

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Background

Measurement of monoclonal immunoglobulin has, to date, been the preferred method for assessment of patients with MM. However, the serum half-life of IgG reduces with increasing concentrations due to saturation of the recycling Fc ("neonatal") receptor (Fig. 1). Determination of the IgG κ / IgG λ ratio would correct for this variable catabolism and potentially provide a sensitive marker of disease. Here we describe nephelometric immunoassays on the Dade-Behring BNTMII, which can determine the serum IgG κ / IgG λ ratio and use these ratios to monitor MM tumour responses to chemotherapy.

Methods

The instrument was programmed to construct a calibration curve from a 6 point, serum based calibration fluid set. The standard curves were validated by assay of serum control fluids. All dilutions were made with the instrument's on-board pipetting system. The main assay characteristics are summarised in the table below:

Assay	IgG κ	IgG λ
Range (g/L)	1.5-48.0	1.0-32.0
Sample dilution	1/20	1/20
Min. sample dilution	1/5	1/5
Sensitivity (g/L)	0.38	0.25
Assay time (mins)	12	12
Intra-assay precision (n=10) %CV (mean)	7.4% (22.63g/L)	2.19% (36.18g/L)
	3.9% (6.28g/L)	5.5% (6.42g/L)

Table 1: Assay parameters of IgG κ / IgG λ assay for use on the Dade-Behring BNTMII

To generate a normal range for the IgG κ / IgG λ ratio 109 normal (blood donor) serum samples were assayed for IgG κ and IgG λ . The patient samples used were archived sera collected from the VIIth UK MRC myeloma trial.

Results

IgG κ and IgG λ concentrations measured in 109 normal (blood donor) serum samples were used for comparisons with patient results; mean IgG κ 6510 mg/L (SD \pm 1368, range 3250 – 10500mg/L), mean IgG λ 5048 mg/L (SD \pm 1441, range 2430-11000), mean IgG κ / IgG λ ratio of 1.35 (SD \pm 0.261, range 0.864-2.022), IgG κ + IgG λ summated well with total IgG (Human IgG Dade-Behring) (Pearsons Correlation 0.8 p>0.01). Presentation samples (Fig. 2) were analysed from 19 patients (10 IgG κ and 9 IgG λ), with serial samples analysis being completed on 9 (4 IgG κ / 5 IgG λ) patients through the course of their disease.

All 19 presentation samples showed elevated concentration of the relevant IgG and abnormal IgG κ / IgG λ ratio. For all samples, (n=25) where immunofixation had been performed and a monoclonal band visible, the IgG κ / IgG λ ratios were abnormal. In 4/4 patients who did not achieve CR the ratio remained abnormal throughout. In 3/5 patient achieving CR IgG κ / IgG λ ratios reported early indication of relapse (Fig 3 / 4.).

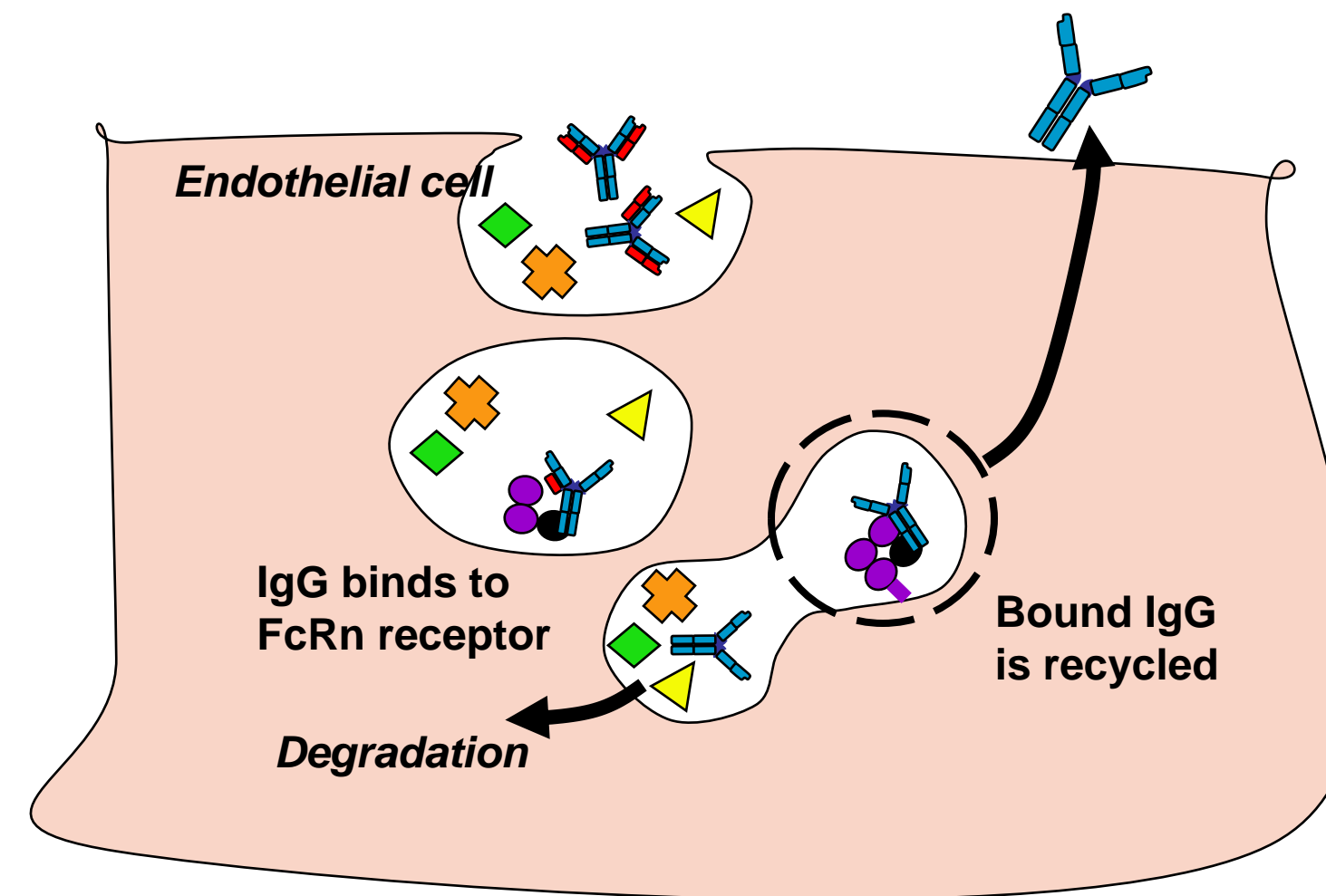


Figure 1: FcRn receptor recycling of IgG in endothelial cells is responsible for the 21 day half life of IgG. This receptor may get saturated at high concentrations of IgG seen in some multiple myeloma patients. Saturation of the FcRn will lead to a decreased recycling rate and decrease the half life of IgG.

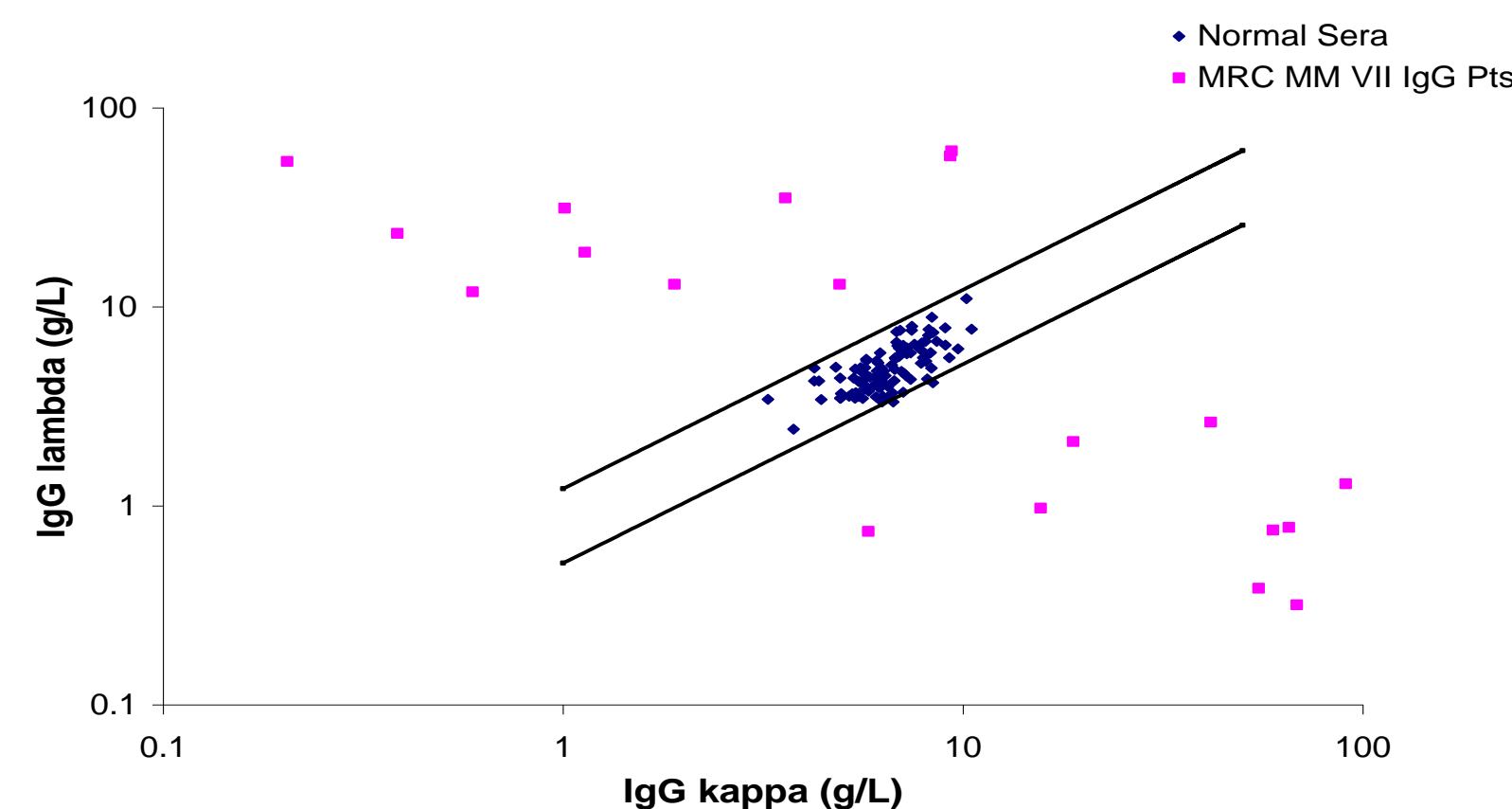


Figure 2: Plot of IgG κ v IgG λ for 109 blood donor sera and 19 presentation sera from MRC Myeloma VII trial. The parallel lines indicates the 95% range for IgG κ / IgG λ ratio.

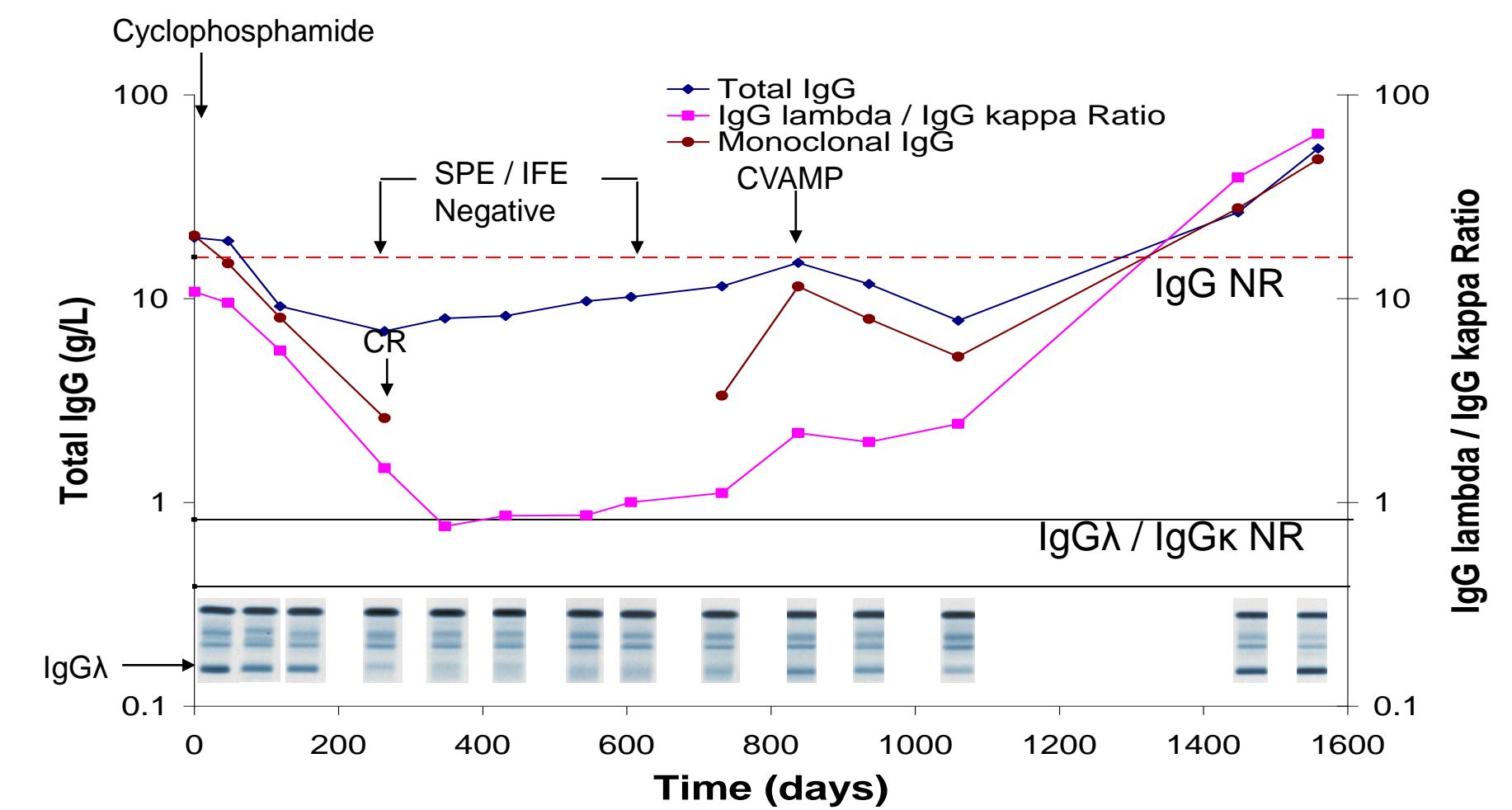


Figure 3: Serial analysis of IgG λ patient sera comparing monoclonal IgG from SPE densitometry, total IgG and IgG λ / IgG κ ratio. The ratio became abnormal before a band was quantifiable by SPE. During the treatment for relapse a fall in total and monoclonal IgG was apparent, the ratio indicated no selective tumour kill.

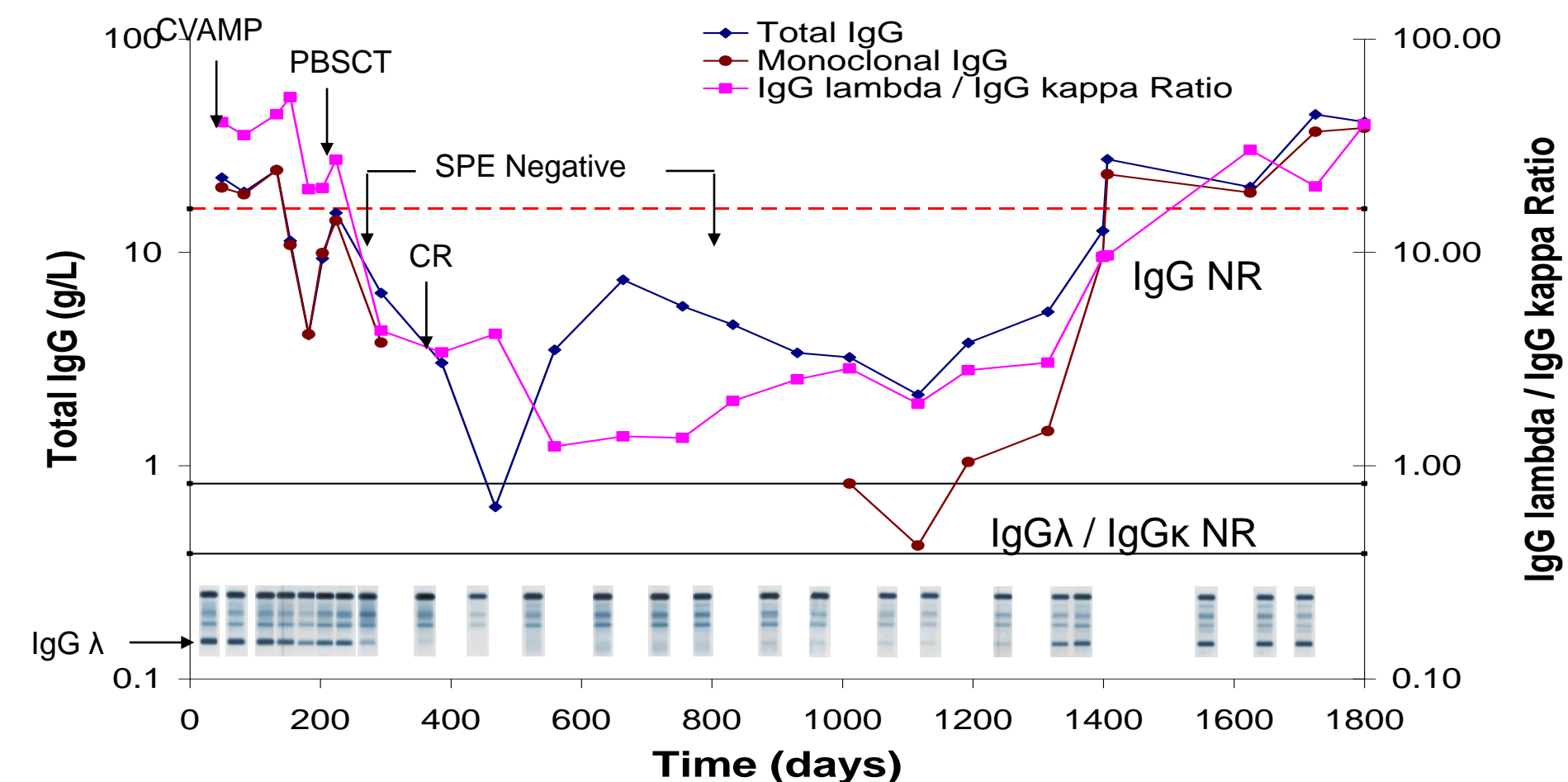


Figure 4: Serial analysis of IgG λ patient sera comparing monoclonal IgG from SPE densitometry, total IgG and IgG λ / IgG κ ratio. The ratio did not normalise even when the patient had been diagnosed as having a complete response. Furthermore, the ratio became increasingly abnormal before a band was quantifiable by SPE

Conclusion

This preliminary data indicates it is possible to type monoclonal immunoglobulins by quantifying the IgG κ / IgG λ ratios. Analysis of this ratio corrects for variable metabolism of IgG, provides quantitative results, and is more sensitive than IFE for the measurement of residual disease in some patients.