anti-HTLV-1 antibody positive and negative cases.

## Discussion

HHV-6 is a new member of the herpes virus group.[1] The predominant affinity of CD4 positive T cells[4] and a high incidence of latent infection with the virus suggest that HHV-6, like other such viruses, may cause specific lymphocyte related disease or may have some role in opportunistic infection. Recently, Josephs *et al* and Jarrett *et al* reported that HHV-6 DNA had been detected in three of 82 B cell lymphomas [8] and two of 117 lymphomas using Southern blot analysis.[7] Buchbinder *et al* and Torelli *et al* also reported detecting HHV-6 DNA in 23 of 25 different malignant lymphomas,[9] three of 25 Hodgkin's lymphomas, and none of 41 non-Hodgkin's lymphomas[6] using PCR methods. A definite association between malignant lymphoma and HHV-6 however, was still unclear.

In our study we found amplified HHV-6 DNA using PCR in 50·0–68·8 % of samples studied, but found no clear differentiation in rates of detection among different types of malignant lymphoma. Using Southern blot analysis, we were unable to detect any HHV-6 DNA. Using PCR, Kondo *et al* identified HHV-6 infected monocyte/macrophage cells in lymphadenopathies.[10] We also clarified that HHV-6 infected CD68-positive monocyte/macrophage cells existed in a latent form, using double staining in situ hybridisation and immunohistochemistry methods.[11] We therefore deduced that the presence of HHV-6 DNA shown by PCR in lymphoid malignancies was derived from latent infection. In this study we were unable to determine the HHV-6 positive cell subsets, but the HHV-6 positive cells might be infected in monocyte/macrophage cells as they are the same as in benign lymphadenitis.

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# Inaccurate haemoglobin estimation in Waldenström's macroglobulinaemia: unusual reaction with monomeric IgM paraprotein

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## Abstract

**Automated blood counts from a patient with Waldenström's macroglobulinaemia repeatedly failed critical limit standards set for mean cell haemoglobin concentration and mean cell haemoglobin. Haemoglobin estimation was higher than that suggested by clinical examination, symptoms, and the spun haematocrit. This was found to be due to an interaction between the Coulter lysing agent and monomeric IgM paraprotein in the patient's plasma, creating a precipitate which was optically dense at 525 nm.**

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Automated blood analyses have greatly increased the availability and accuracy of full blood counts. Haemoglobin measurements are made by converting haemoglobin to cyanmethaemoglobin and then measuring absorbance at 525 nm. The mean cell

*Estimation of OD at 525 nm of IgM and IgG fractions by Quick-Sep separation with and without addition of Lyse-S in test and control plasmas*

| *Plasma fraction* | *Sample* | *OD at 525 nm* | |
|---|---|---|---|
| | | *- Lyse-S* | *+ Lyse-S* |
| IgG | Patient | 0·072 | 2·250 |
| | IgG myeloma | 0·056 | 0·060 |
| | Waldenström's macroglobulinaemia control | 0·083 | 0·223 |
| IgM | Patient | 0·062 | 0·067 |
| | IgG myeloma | 0·074 | 0·082 |
| | Waldenström's macroglobulinaemia control | 0·064 | 0·106 |

haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) are derived from the directly measured haemoglobin, red cell count, and mean cell volume.[1] Other factors which alter the optical density of a blood sample, such as lipaemia or high white cell counts, are well recognised to confound haemoglobin estimations using this technique.[1]

It was noted in our laboratory that samples from a patient with an IgM secreting low grade non-Hodgkin's lymphoma (Waldenström's macroglobulinaemia) repeatedly failed critical limits for both MCHC and MCH using Technicon H6000, Coulter JS, and Coulter STKR automated cell counters.

Clinical symptoms and manual haematocrits, performed by a spin technique, indicated that the patient was more anaemic than was suggested by the reported haemoglobin concentration. If the plasma from the sample was removed and replaced by an equal volume of Isoton, the measured haemoglobin was 40 g/l lower than first estimated. Treatment of the plasma with 2-mercaptoethanol (2ME) did not correct this discrepancy. No cryoglobulin could be detected.

## Method and Results

The patient's plasma was separated from fresh EDTA samples, mixed with Coulter lysing solution (Lyse-S), and analysed through a Coulter STKR. The plasma treated in this way gave a "haemoglobin" estimation of 40 g/l, confirming that a plasma fraction was interacting with the lysing reagent to cause the false reading. The patient's plasma was then separated into IgG and IgM fractions using Quick-Sep columns, according to the manufacturer's protocol (Isolab Inc, Drawer 4350, Akron, Ohio, USA). As controls, plasma was obtained from a known IgG myeloma and from another patient with Waldenström's macroglobulinaemia in whom haemoglobin estimates were felt to be accurate and these were separated in the same way. The optical density (OD) of each fraction was then measured at 525 nm before and after the addition of Lyse-S at a 1 in 7 dilution. A sharp increase in OD of the test IgG fraction was seen. There was also a small increase in the OD of the IgG fraction from plasma from the control with Waldenström's macroglobulinaemia but no change in the IgG myeloma control (table).

A precipitate formed when the patient's IgG fraction was incubated at 4°C. The precipitate was separated from the supernatant fluid by centrifugation, washed twice in pH 8·6 buffer, and resuspended. The supernatant fluid, the resuspended precipitate, and the Ig fractions from Quick-Sep separation were electrophoresed on agarose at pH 8·6, and immunofixation, using anti-IgG and anti IgM, was performed. This showed that IgM could be detected in both the IgG and IgM fractions of the patient's plasma and in the precipitate. IgG was present in the plasma IgG fraction and in the supernatant fluid, but none could be detected in the precipitate.

## Conclusion

It is concluded that there is a reaction between Lyse-S and the paraprotein which passed with the IgG fraction in Quick-Sep filtration. This results in an increase in plasma OD and consequently in inaccurate haemoglobin estimation. In view of its mobility with the IgG fraction and the lack of removal with 2ME, this is likely to represent monomeric IgM.

Patients with Waldenström's macroglobulinaemia are known to produce non-pentameric IgM components in varying amounts,[2,3] probably because of defective polymerisation.[4] No similar reactions have been described before, but the findings of small increases in the OD of the plasma fractions in the patient with Waldenström's macroglobulinaemia selected as a control suggests that the difference may be one of degree; the more monomeric IgM formed, the greater the chance of detecting similar interactions. This would merit further assessment.

In the case described, in order to obtain an accurate haemoglobin measurement for clinical use, all the plasma has to be removed from a spun EDTA sample. This is replaced with an equal volume of Isoton and the manipulated sample is then processed through the Coulter STKR in the normal manner.

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