Lumbar puncture (LP) still has an important role to play in the diagnosis of subarachnoid hemorrhage. Although computed tomography (CT) scanning has replaced LP as the investigation of first choice, and LP was a useful test in the investigation of first choice, within 24 h of ictus 5% of patients with equivocal values with net bilirubin absorbances from 0.005 to 0.014.

In our control samples, net bilirubin absorbances ranged from 0 to 0.007, with a mean of 0.0023. The five patients with absorbances >0.015 all had subarachnoid hemorrhage: four because of aneurysmal rupture (proven angiographically) and one because of severe head injury. In our experience, at least three of these patients had a negative result for xanthochromia by direct inspection. HB absorbances of these specimens, with net bilirubin absorbances in parenthesis, were 0.022 (0.002), 0.047 (0.026), 1.27 (0.93), 0.22 (0.18), and 0.2 (0.048). Thus, the HB absorbances were variable and in four of the five samples were no different to those found in control specimens (range, 0–0.35; mean, 0.077 ± 0.1 SD).

Seven patients had net bilirubin absorbances between 0.005 and 0.014. Of these, four showed no abnormalities present by CT head scans and cerebral angiography. The other three did not undergo angiography, but were considered by a consultant neurologist to have migraine. At this time, we report values between 0.01 and 0.015 as borderline positive and values >0.015 as positive.

The scans of six patients shown in Fig. 1 were typical of the scans obtained. In patients A and C, considerable HB was present, but only patient C was reported and confirmed clinically as positive for xanthochromia. With patient B, the 415/440 ratio was close to unity, indicating almost pure bilirubin, but the absorbances were low enough to consider this patient’s result as being negative. In patients D, E, and F, although the absorbances of HB were similar (0.022), the net bilirubin absorbances and consequent clinical reports were different: 0.006 (negative), 0.003 (negative), and 0.022 (positive), respectively.

The report that one can use a CSF HB absorbance at 415 nm of >0.023 as a possible index of intracranial hemorrhage (3) is not tenable. For example, in one of our positive samples, the absorbance at 415 nm was 0.022. In addition, the finding that CSF proteins >1 g/L can produce absorbances >0.023 at 415 nm would suggest that absorbance measurements solely at 415 nm would yield low sensitivity in relation to determining xanthochromia (4). It makes good sense, therefore, to do a full spectral scan because it is the spectral hump between 440 and 460 nm that is clinically significant for xanthochromia.

One CSF specimen kept at room temperature (23 °C) for 48 h gave absorbances for HB of 0.03, 0.09, and 0.31 at 0, 24, and 48 h, respectively. The absorbance due to
bilirubin was 0 over these 3 days, suggesting that CSF
does not need to be analyzed immediately, because there
was no detectable alteration of Hb to bilirubin within the
CSF after collection. This is consistent with the finding
that Hb requires the enzyme hemoglobin oxidase present
in macrophages within the arachnoid and choroid plexus
to be converted to bilirubin (5).

Addition of bilirubin (Sigma Chemical Co.) in increasing
amounts to CSF (net bilirubin absorbances, 0.024–0.3)
yielded a recovery of bilirubin calculated by this method
of 98.2% (± 6.0%, n = 9). A quality-control sample was
prepared by diluting serum 100-fold in water and adding
a known amount of bilirubin. The interday CV of this
quality-control sample analyzed by three separate ana-

Fig. 1. Spectrophotometric scans on CSF specimens from six patients with suspected intracranial hemorrhages.
Drawn tangents (vertical lines) are the absorbances at 415 and 440 nm.
lysis 13 times over a 1-month period was 6.0% (mean net bilirubin absorbance, 0.0346 ± 0.0021). The intraday CV was 3.3% on this sample (mean absorbance, 0.0377 ± 0.00123, n = 12).

The correlation between serum bilirubin and CSF bilirubin was −0.058 (n = 31), indicating that the CSF bilirubin absorbance was not because of diffusion of bilirubin into the CSF or a consistent contamination of the CSF with vascular blood during the LP. In all our positive results to date, the clinical follow up has confirmed positive xanthochromia.

In conclusion, this test is simple, inexpensive, can easily be interpreted, and thereby overcomes the confusion in diagnosing subarachnoid hemorrhage (6–8). We have used it for the last 20 months in over 50 patients, and it has proven clinically useful.

References

Interferences with Two-site Immunoassays by Human Anti-mouse Antibodies Formed by Patients Treated with Monoclonal Antibodies: Comparison of Different Blocking Reagents, Jochen Reinsberg (Zentrum für Frauenheilkunde und Geburtshilfe, Universität Bonn, Sigmund-Freud-Strasse 25, D-53127 Bonn, Germany; fax 49-228-2874651)

The most widely used approach for reducing interferences with two-site immunoassays by human anti-mouse antibodies (HAMAs) developed by many patients after exposure to murine immunoglobulins is to include high amounts of nonspecific mouse IgG within the assay buffer (1, 2). Recently, Mössner et al. (3) have developed a polymerized form of murine IgG (MAK33), which they found to be superior to normal mouse IgG for blocking HAMA interferences. In contrast, we observed false-positive values for the cancer antigen 125 (CA 125), because of HAMA interferences in samples from ovarian cancer patients treated with the anti-CA-125 antibody OC125, which could be corrected by preincubation with mouse IgG but not with MAK33 (4). The aim of the present study was to examine whether treatment with other monoclonal antibodies gives rise to additional HAMAs that are insensitive to MAK33 and to further characterize this specific HAMA response.

Sixty-four serum samples were obtained from 51 ovarian cancer patients who, in the course of several clinical studies (5–8), had received multiple infusions of one of four murine monoclonal antibodies: OC125 (16 patients), B43.13 (8 patients), ACA125 (18 patients), and B72.3 (9 patients). The procedures followed in this study were in accordance with the standards of the ethical committee of our faculty. The interfering HAMA activity of the samples was quantified with a bridging HAMA assay (HAMA-ELISA medac, Medac) involving polyclonal murine IgG in both control and detection steps before and after preincubation (30 min at room temperature) with the following: (a) polyMAK-33 (MAK33), a polymerized murine IgG1 preparation (gift of Boehringer Mannheim, Mannheim, Germany); (b) Immunoglobulin Inhibiting Reagent (IIR) a formulation of immunoglobulins targeted against HAMAs (gift of Bioreclamation, East Meadow, NY); (c) polyclonal mouse IgG (reagent grade, Sigma Chemical Co.); and (d) mouse IgG of different subclasses, purified from myeloma proteins (Sigma) of the plasmacytoma cell lines UPC-10, HOPC-1, MOPC-141, and FLOPC-21.

Comparison of blocking reagents. In 35 samples, the interfering HAMA activity (ranging from 1162 to 55 397 µg/L) could be blocked completely by each of the three blocking agents (HAMA activity <100 µg/L after preincubation with 0.7 g/L MAK33, 2 g/L IIR, or 0.7 g/L polyclonal mouse IgG). In contrast, in 29 samples, the interfering HAMA activity (ranging from 1160 to 1 648 000 µg/L) could be eliminated only by IIR and polyclonal mouse IgG, respectively, whereas after preincubation with MAK33, increased HAMA values were still measured (Fig. 1). Residual HAMA activity insensitive to MAK33 was observed in most samples obtained from patients treated with OC125 fragments (16 of 18) compared with a considerably lower incidence in the other treatment groups (5 of 16 treated with B43.13, 4 of 19 treated with ACA125, and 4 of 11 treated with B72.3).

Specificity of the HAMA response insensitive to MAK33. To characterize the specificity of the HAMAs insensitive to MAK33 and to clarify how far they are directed against isotypic determinants not expressed on the gamma, heavy or the kappa light chain presented by MAK33, six samples (three OC125 patients, two ACA125 patients, and one B72.3 patient) were preincubated with supplementary 0.3 g/L murine IgG of different subclasses in the presence of 0.7 g/L MAK33. Nearly complete inhibition (73–99%) was achieved in all six samples by the IgG2a antibody UPC-10, whereas the IgG2a antibody HOPC-1 and the IgG3x antibody FLOPC-21 were ineffective in two samples, and in two samples only weak inhibition (17–35%) was observed. This indicates that the HAMAs of at least four samples bind to epitopes on the UPC-10 antibody.