Why Human Color Vision Cannot Reliably Detect Cerebrospinal Fluid Xanthochromia

Axel Petzold, MD, PhD; Geoffrey Keir, PhD, MSc FRCPath FIBMS; Ted L. Sharpe, PhD, MA

Background—Visual assessment of cerebrospinal fluid (CSF) for xanthochromia (yellow color) is practiced by the majority of laboratories worldwide as a means of diagnosing intracranial bleeds.

Methods—Colorimetric and spectrophotometric analysis of CSF samples for recognizing the presence of bilirubin either in low concentrations or in the presence of hemolysed blood.

Results—The experiments provide the physiological and colorimetric basis for abandoning visual assessment of CSF for xanthochromia.

Conclusion—We strongly recommend relying on spectrophotometry as the analytical method of choice. (Stroke. 2005;36:1295-1297.)

Key Words: cerebrospinal fluid ■ intracerebral hemorrhage ■ subarachnoid hemorrhage

Subarachnoid hemorrhage is one of the most striking conditions in medicine with potentially fatal outcome. The analysis of cerebrospinal fluid (CSF) is a crucial diagnostic tool. The sensitivity for detecting a bleed by CT decreases from 95% on day 1 to <10% 3 weeks after the event, whereas the sensitivity of CSF analysis remains close to 100%. The presence of pigments in CSF alters its visual appearance. Oxyhemoglobin makes it appear red or orange, whereas bilirubin gives the yellow coloration of true xanthochromia. Oxyhemoglobin arises both from a traumatic tap and a true subarachnoid hemorrhage. Importantly, the conversion of oxyhemoglobin to bilirubin can only happen in vivo, allowing distinction between a true intracranial bleed and one caused by a traumatic tap. Here, we provide physiological evidence that the commonly practiced visual assessment of CSF should be abandoned and replaced by spectrophotometry.

Materials and Methods
To simulate the conditions in which bilirubin may be observed, a set of experiments were designed. First, to reproduce contamination by oxyhemoglobin as it may occur with a traumatic tap, a series of tubes containing doubling dilutions of hemolysed blood (series A inset in Figure 1A) into CSF containing the same amount of bilirubin was prepared. Second, to determine the lowest concentration of bilirubin that could be confidently detected, we prepared a series of tubes containing doubling dilutions of bilirubin alone (series B inset in Figure 1A).

All tubes were examined visually for xanthochromia, in normal daylight or cool white fluorescent light, the typical viewing conditions, by 11 analysts, comprising clinical scientists, biomedical scientists, or clinical neurology staff within the Department of Neuroimmunology at National Hospital for Neurology and Neurosurgery. The tubes were presented in a random order and the analysts were naive to their actual concentrations.

Once the visual assessments were complete, all samples were scanned between 350 and 740 nm using an Ultrospec 4300 pro (Amersham Biosciences). The same analysts were then asked to indicate whether bilirubin was present or absent in the scan. The proportions of subjects finding a positive result by visual or spectroscopic assessment were compared using a χ² test.

Finally, the x–y chromaticity coordinates of the spectrophotometric scans were calculated according to the standard procedures of specification for visual assessment established by the Commission Internationale de l’Eclairage (CIE, the International Commission on Illumination). This involves multiplying the spectral transmittances of the samples, converted from their optical densities, by the spectral concentration of the radiant power of the source illuminating them and then multiplying the product by each of the 3 color-matching functions, which define the CIE standard colorimetric observer. The resulting x–y chromaticity coordinates of the samples can then be plotted in the CIE 1931 chromaticity diagram for the standard 2° field of view (Figure 1A and 1B), and their dominant wavelengths, which correspond to hue, and excitation purities, which correspond to saturation, can be geometrically calculated (see explanations in the Table and Figure legends and values in Table). The calculations were made for 2 CIE illumination or lighting standards: “D65,” which equates to average daylight; and “A,” which is for tungsten light.

Results
Samples in series A, when calculated for viewing by standard illuminant D65, varied in their dominant wavelength from 572 nm (which falls near the hue category “pure yellow”) to 615 nm (red). They also varied in their...
excitation purity from 97.9% (highly saturated) to 34.1% (moderately saturated). In contrast, the samples in series B all had the same dominant wavelength, 572 nm (yellow), but differed in their excitation purity from 0.62 (very desaturated) to 36.6% (Table). The optical density for bilirubin (450 to 460 nm) ranged from 3.5 to 0.36 for samples A1 to A14 and from 3.2 to 0.002 for samples B1 to B8.

A significantly higher proportion of the analysts detected traces of bilirubin spectrophotometrically than visually, both when the xanthochromic CSF samples were contaminated by the presence of hemolyzed blood (series A) and when they were desaturated (series B). In series A, visual detection failed for CSF samples with dominant wavelengths >574 nm (samples A1 to A7), most of which fall considerably outside the color category “pure yellow.” In series B, bilirubin could not be reliably detected in CSF specimens with excitation purity levels <2.4% (samples B5 to B8). In contrast, in both series A and B, bilirubin could be reliably detected in all the samples by examining the spectrophotometric scans.

This study confirms that spectrophotometry is superior to color vision for analyzing CSF samples for the presence of bilirubin. Most critical CSF samples are either contaminated by oxyhemoglobin or have only low levels of bilirubin. Under such conditions, detection of xanthochromia becomes unreliable, especially when viewed under incandescent lighting or a tungsten desk lamp (corresponding to CIE standard illuminant A). A lower proportion of the assessors were able to detect xanthochromia for samples B4 (7/11, $\chi^2=4.88, P<0.05$), B5 (3/11, $\chi^2=9.21, P<0.01$), and B6 (0/11, $\chi^2=6.47, P=0.01$; see insets in Figure 1B) under tungsten light than under daylight conditions. Colorimetric analysis revealed that all of the samples now fell completely outside the “pure yellow” category. This condition represents a “worst-case scenario,” such as may be encountered during a night on-call.

**Discussion**

The implications of these findings can be judged from our previous analysis of spectrophotometric scans of CSF samples, which did not appear yellow in almost 80% of the cases encountered at the National Hospital for Neurology and Neurosurgery. Approximately 80% of all CSF samples with significant amounts of bilirubin appear rather “red” than “yellow,” but 99.7% of >3500 laboratories participating in 2 recent American surveys still assess samples by color vision. The observations presented here provide a physiological basis for abandoning the visual assessment of CSF for xanthochromia and rely on spectrophotometry instead.

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**Table**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chromaticity-Coordinates</th>
<th>Dominant wavelength (nm)</th>
<th>Excitation Purity (%)</th>
<th>Color Vision (observed frequency)</th>
<th>Spectrophotometry (observed frequency)</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
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<tbody>
<tr>
<td>A1</td>
<td>0.3824, 0.3990</td>
<td>615</td>
<td>97.9</td>
<td>0.00</td>
<td>0.72</td>
<td>12.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A2</td>
<td>0.3828, 0.3993</td>
<td>607</td>
<td>91.2</td>
<td>0.00</td>
<td>0.72</td>
<td>12.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A3</td>
<td>0.3836, 0.4002</td>
<td>596</td>
<td>77.3</td>
<td>0.00</td>
<td>0.72</td>
<td>12.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A4</td>
<td>0.3847, 0.4011</td>
<td>586</td>
<td>64.3</td>
<td>0.00</td>
<td>0.72</td>
<td>12.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A5</td>
<td>0.3868, 0.4030</td>
<td>580</td>
<td>55.4</td>
<td>0.09</td>
<td>0.72</td>
<td>9.21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A6</td>
<td>0.3903, 0.4058</td>
<td>576</td>
<td>48.0</td>
<td>0.18</td>
<td>0.90</td>
<td>11.73</td>
<td>&lt;0.001</td>
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<tr>
<td>A7</td>
<td>0.3961, 0.4097</td>
<td>574</td>
<td>42.8</td>
<td>0.63</td>
<td>1.00</td>
<td>4.88</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A8</td>
<td>0.4052, 0.4138</td>
<td>573</td>
<td>39.5</td>
<td>1.00</td>
<td>1.00</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>A9</td>
<td>0.4209, 0.4166</td>
<td>572</td>
<td>36.7</td>
<td>1.00</td>
<td>1.00</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>A10</td>
<td>0.4478, 0.4142</td>
<td>572</td>
<td>36.0</td>
<td>1.00</td>
<td>1.00</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>A11</td>
<td>0.4922, 0.4000</td>
<td>572</td>
<td>34.8</td>
<td>1.00</td>
<td>1.00</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>A12</td>
<td>0.5619, 0.3709</td>
<td>572</td>
<td>34.6</td>
<td>1.00</td>
<td>1.00</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>A13</td>
<td>0.6354, 0.3383</td>
<td>572</td>
<td>34.3</td>
<td>1.00</td>
<td>1.00</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>A14</td>
<td>0.6769, 0.3180</td>
<td>572</td>
<td>34.1</td>
<td>1.00</td>
<td>1.00</td>
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<td>NS</td>
</tr>
<tr>
<td>A15</td>
<td>0.3883, 0.4052</td>
<td>572</td>
<td>36.6</td>
<td>1.00</td>
<td>1.00</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>B1</td>
<td>0.3615, 0.3702</td>
<td>572</td>
<td>18.2</td>
<td>1.00</td>
<td>1.00</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>B2</td>
<td>0.3474, 0.3518</td>
<td>572</td>
<td>9.3</td>
<td>1.00</td>
<td>1.00</td>
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</tr>
<tr>
<td>B3</td>
<td>0.3403, 0.3425</td>
<td>572</td>
<td>4.8</td>
<td>0.90</td>
<td>1.00</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>B4</td>
<td>0.3366, 0.3378</td>
<td>572</td>
<td>2.4</td>
<td>0.45</td>
<td>1.00</td>
<td>8.25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B5</td>
<td>0.3348, 0.3354</td>
<td>572</td>
<td>1.66</td>
<td>0.00</td>
<td>1.00</td>
<td>22.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B6</td>
<td>0.3338, 0.3341</td>
<td>572</td>
<td>1.04</td>
<td>0.00</td>
<td>1.00</td>
<td>22.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B7</td>
<td>0.3334, 0.3336</td>
<td>572</td>
<td>0.62</td>
<td>0.00</td>
<td>1.00</td>
<td>22.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NS indicates not significant.

Color specifications (CIE 1931 x-y chromaticity coordinates, dominant wavelengths and excitation purities), calculated with respect to CIE illuminant D65, of 14 samples derived by double diluting hemolyzed blood (Series A) and 8 samples derived from a double dilution of xanthochromic CSF in H2O (Series B). The dominant wavelengths [5] were geometrically derived by drawing lines from the white-point (D65) through the x-y chromaticity coordinates of the samples to the corresponding spectral wavelengths indicated on the outer boundary (spectral locus) of the chromaticity diagram (Figure 1). The excitation purities [5] are the ratios of distances in the chromaticity diagram, indicating how far the chromaticity points of the samples are displaced from the white-point towards the spectral locus. Also shown are the observed frequencies of assessors who correctly identified the presence of bilirubin in the vision and spectrophotometry experiments and the results of the $\chi^2$ analysis.

References


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