Spectrophotometry of cerebrospinal fluid in subacute and chronic subdural haematomas

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SYNOPSIS Spectrophotometric examinations were performed on cerebrospinal and subdural fluids in subacute (five patients) and chronic (20 patients) subdural haematomas, with special reference to the diagnostic aid of CSF spectrophotometry. Spectrophotometric xanthochromia of haemorrhagic origin was found in all CSFs examined, while definite visible xanthochromia was observed in only 28% and the CSF was judged as colourless in 52% of those cases. Characteristic bleeding patterns were found spectrophotometrically in all the 20 CSFs examined within 24 hours after lumbar puncture, haematoma patterns being detected in 90–95% of the cases. In many cases the electrophoretically separated protein fractions of CSF and subdural fluids were spectrophotometrically examined. In conclusion, CSF spectrophotometry is a simple, fast, and extremely sensitive method, which in our opinion should be used routinely in the diagnosis of suspected subdural haematomas, if lumbar puncture is not contraindicated.

The diagnosis of subdural haematoma is often very difficult. A correct diagnosis is, however, very important for a prompt neurosurgical treatment of the disorder. Increased diagnostic aids are therefore of the utmost importance.

Recent investigations (Kjellin, 1969, 1971a, b, c) have shown that the xanthochromic substances in the cerebrospinal fluid (CSF) are bound in a non-ultrafilterable form, obviously as protein compounds, the CSF bilirubin including several compounds (LB- and SB-compounds, direct-diazoreacting and indirect-diazoreacting bilirubinoids), and that the methaem-compounds also include methaemalbumin. The non-ultrafilterable form of the xanthochromic compounds in the CSF makes it possible to concentrate them by ultrafiltrating the CSF, and thus detect them even in very low concentrations—for example, in infarcts of the central nervous system and subdural haematomas (Kjellin, 1970, 1971a).

Electrophoretically, the bilirubinoids are recovered in the albumin fraction (infrequently in the prealbumin fraction) together with methaemalbumin; on the other hand, oxyhaemoglobin and methaemoglobin compounds are found in

the beta₁-globulin fraction of the CSF (Kjellin, 1969, 1971a). By isoelectric focusing of the CSF the xanthochromic fractions found upon electrophoresis can be further subdivided (Kjellin, 1972, 1973).

In vascular diseases of the nervous system, the appearance of xanthochromic compounds with different substance patterns can be demonstrated in the CSF by spectrophotometry (see Kjellin, 1969, 1970, 1971a, b, c, 1972, including references). The diagnostic significance of CSF spectrophotometry in cerebrovascular diseases has recently been reported by Kjellin and Söderström (1972, 1973, 1974).

Barrows *et al.* (1955) examined the subdural fluids from eight patients with subacute or chronic subdural haematomas. Methaemoglobin was found in all fluids, and bilirubin in addition in six of the subdural fluids. In none of their cases was methaemoglobin observed in the CSF, the authors concluding that the yellow colour of CSF in chronic subdural haematomas had been due to bilirubin in the few random observations that had been made.

Kronholm and Lintrup (1960) reported seven cases of subdural haematomas (acute or chronic not indicated) in which the light absorption

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curve of CSF was elevated in all cases, showing a maximum in the vicinity of 406 nm. Frequently, however, the increase was only slight according to the authors.

Kjellin and Steiner (1971) in a preliminary report described the spectrophotometric findings in chronic subdural haematomas. Xanthochromic compounds (bilirubin, oxyhaemoglobin, methaemalbumin and/or methaemoglobin compounds) of haemorrhagic origin were found in the CSF from all the eight patients examined. Met-haem compounds were observed in four of the eight CSFs.

The present investigation was performed on an extended series of patients, including subacute and, predominantly, chronic subdural haematomas, in order to define the diagnostic aid of CSF spectrophotometry. Furthermore, the corresponding subdural-haematoma fluids were examined in a similar way as the CSF. The figures of frequency of spectrophotometric patterns found in CSF and subdural fluids were determined. The protein fractions found upon electrophoresis of CSF and subdural fluids were examined spectrophotometrically in many cases.

MATERIAL AND METHODS

The twenty-six patients included 20 cases with 'chronic' subdural haematomas, five cases with 'subacute' subdural haematomas, and one case with epidural haematoma admitted to the Karolinska Hospital in 1971-73. Acute subdural haematomas associated with cranial injuries have thus not been included. In such cases the spectrophotometric CSF-findings are dominated by the pattern of a subarachnoid bleeding (Kjellin, 1970).

The results of the CSF examinations were checked by one of the authors (Kjellin), while the co-author (Steiner) scrutinized the other clinical findings.

The CSF samples were collected close to the

TABLE 1
PATHOLOGICAL SPECTROPHOTOMETRIC PATTERNS

<i>Spectrophotometric pattern found in cases with</i>	<i>Symbols</i>	<i>Characteristic features</i>
Encapsulated haematoma	H	Mainly methaem-compounds (methaemalbumin and methaemoglobin compounds)
Subarachnoid haemorrhage	S	Oxyhaemoglobin and/or bilirubin derived from haemorrhage
Haematoma with subarachnoid haemorrhage	H + S (or S + H)	Mixed pattern of H and S, initial symbol indicating the dominating factor

following operation, and representative samples of the subdural fluids were taken at the operation. Two consecutive samples of CSF were withdrawn at the lumbar puncture in order to determine any traumatic lumbar puncture bleeding (Hellström and Kjellin, 1971; Kjellin, 1971a).

CSF spectrophotometry was carried out as described previously by Kjellin (1969; 1971a, b). The

TABLE 2
CSF FINDINGS IN 25 CASES OF SUBACUTE AND CHRONIC SUBDURAL HAEMATOMAS, AND ONE CASE OF EPIDURAL HAEMATOMA

<i>Diagnosis and patient (sex/age \ yr)</i>	<i>CSF findings</i>			
	<i>Protein concn (mg/100 ml)</i>	<i>Cells (per 3.2 mm³)</i>	<i>Xanthochromia</i>	<i>Spectrophotometric findings</i>
Subacute subdural haematoma				
K.G. (M/65)	78	74 M	V	S + H
E.L. (M/48)	46	N.n.	V?	H
M.P. (F/66)	37	N.n.	V?	H
A.S. (M/34)	73	N.n.	V	H + S
G.S. (M/60)	82	N.n.	V	H + S
Chronic subdural haematoma				
A.B. (M/31)	46	N.n.	I	S + H
D.B. (M/45)	34	L.p.b.	I	Tr. of haem. comp. (examined 3 weeks after l.p.)
E.R. (M/68)	94	L.p.b.	V	H + S
O.B. (M/68)	50	L.p.b.	I	Tr. of haem. comp. (examined 4 days after l.p.)
R.B. (M/52)	39	N.n.	I	Tr. of haem. comp. (examined 4 days after l.p.)
I.C. (M/71)	96	N.n.	V	S + H
S.D. (M/44)	316	L.p.b.	V	S ('old' appearance)
M.G. (F/72)	61	N.n.	I	S + H
A.H. (M/70)	55	26M, 8P	I	S + H
G.L. (M/64)	36	N.n.	I	H
P.L. (M/52)	78	26M, 2P	V?	H
S.Ma. (M/61)	58	N.n.	I	H + S
J.M. (M/73)	92	N.n.	I	H
S.Mo. (M/63)	63	N.n.	I	Tr. of haem. comp. (examined 4 days after l.p.)
G.M. (M/50)	97	N.n.	V?	S + H?
B.N. (M/35)	55	N.n.	I	S + H
B.P. (M/40)	56	N.n.	I	S + H
T.R. (M/67)	46	N.n.	V	H
S.S. (F/70)	88	N.n.	V?	S + H
M.W. (F/16)	27	N.n.	I	S + H (CSF conc. × 10, examined 5 days after l.p.)
Epidural haematoma				
I.R. (F/36)	59	N.n.	I	Tr. of haem. comp.

For explanations of the symbols of the spectrophotometric patterns see Table 1.

Abbreviations used: I = Invisible xanthochromia; V = visible xanthochromia; V? = visible xanthochromia questionable; N.n. = nothing noteworthy; L.p.b. = lumbar puncture bleeding; M = monocytes, and P = polynuclear white cells; Tr. of haem. comp. = traces of xanthochromic compounds of haemorrhagic origin.

spectrophotometric findings were corrected for traumatic puncture bleeding as mentioned above. The CSF examinations also included cell examination and determination of protein concentration. The cells in the CSF were examined by routine microscopy and counted per 3.2 mm^3 . The CSF-protein concentration was determined by a modification of the method of Lowry *et al.* (1951).

In many cases electrophoretic examinations were performed on the CSF and the corresponding

serum, and in some of those electrophoreses the protein fractions were spectrophotometrically examined as described by Kjellin (1969).

In most cases the subdural-haematoma fluids were then examined in a manner similar to the corresponding CSF samples. However, the protein and spectrophotometric examinations of the subdural fluids were performed after diluting the fluids with physiological NaCl.

The abnormal spectrophotometric patterns found

FIG. 1. Characteristic bleeding patterns (B-D) found by spectrophotometry. For explanation of the symbols of the spectrophotometric patterns: see Table 1. Spectrophotometric findings in normal CSF (A1, A2) given for comparison. Direct-spectrophotometry performed with a Beckman DB-G spectrophotometer with scale expander. Wavelength in nm on the abscissa. A1. Normal CSF. A2. The same CSF, scale expansion '10'. B1. H-pattern. B2. A detail study of B1, scale expansion '5'. C1. Mixed pattern (H+S). C2. A detail study of C1, scale expansion '5'. D1. S-pattern of 'recent' appearance. D2. S-pattern of 'old' appearance (as found in CSF more than a week after subarachnoid haemorrhage).

were labelled with the symbols used by Kjellin and Söderström (1974). The characteristic features of the patterns are given in Table 1, and illustrated by the spectrophotograms reproduced in Fig. 1.

RESULTS

The CSF findings in the 26 cases examined are given in Table 2. In five of the cases the CSF was not examined spectrophotometrically until four to five days (four cases) and even three weeks (one case) after the lumbar puncture. In these five cases a definite spectrophotometric pattern (S+H) could be observed in only one case in which ultrafiltered CSF was examined five days after the lumbar puncture, while only traces of haemorrhagic compounds were found in the other four. The CSF had been judged as colourless at the lumbar puncture in all five cases. Those findings confirm previous observations (Kjellin, unpublished data) that colourless CSF samples with 'spectrophotometric' xanthochromia should be examined within one or two days, preferably within 24 hours, after the lumbar puncture.

The spectrophotometric CSF findings in the 20 cases of subdural haematoma examined within 24 hours after lumbar puncture are summarized in Table 3a. Mixed patterns (H+S, or S+H) were most frequently found (60–65% of the cases) while H-pattern was observed in 30% of the cases. S-pattern (of 'old' appearance with only bilirubin) was found in only one patient. It must here be mentioned that the CSF from

those 20 subjects had been judged as colourless in 40% of the cases, while a definite xanthochromia was observed in 35%. In 25% of the cases the visible xanthochromia of the CSF was questionable.

In one examined case with epidural haematoma only traces of haemorrhagic compounds were found without any definite spectrophotometric pattern.

Quantitative paper electrophoresis of the CSF was performed in 12 of the 20 cases of subdural haematoma in which CSF spectrophotometry had been carried out within 24 hours after lumbar puncture. Barrier-damage patterns (slight, moderate, or pronounced) were found in all those cases, combined with increased CSF-beta₁-

TABLE 4

SPECTROPHOTOMETRIC FINDINGS IN CSF AND CORRESPONDING SUBDURAL FLUIDS: 24 SUBDURAL FLUIDS WERE EXAMINED FROM 23 PATIENTS, FLUIDS FROM DOUBLE-SIDED HAEMATOMAS BEING EXAMINED IN ONE CASE

<i>Diagnosis, patient</i>	<i>Spectrophotometric findings</i>		<i>Protein concn. in subdural fluid (mg/100 ml)</i>
	<i>CSF</i>	<i>Subdural fluid</i>	
Subacute subdural haematoma			
K.G.	S + H	S	6,800
E.L.	H	H	5,800
M.P.	H	H	Not examined
A.S.	H + S	H	7,000
G.S.	H + S	H	4,600
Chronic subdural haematoma			
A.B.	S + H	H + S	5,400
E.B.	H + S	H	4,300
O.B.	Tr. of haem. comp.	H + S	4,900
R.B.	Tr. of haem. comp.	H + S	5,900
I.C.	S + H	H	Not examined
S.D.	S	H	7,100
M.G.	S + H	H + S	4,400
A.H.	S + H	H	2,900
G.L.	H	H	8,800
P.L.	H	H	6,700
S.Ma.	H + S	H + S	5,400
S.Mo.	Tr. of haem. comp.	S + H	4,400
G.M.	S + H?	S (left haema- toma) H + S (right haematoma)	6,000 4,600
B.N.	S + H	H	4,500
B.P.	S + H	S + H	940
T.R.	H	H + S	4,500
S.S.	S + H	S + H	4,400
M.W.	S + H	S + H	4,400

TABLE 3

a. SPECTROPHOTOMETRIC CSF FINDINGS IN 20 CASES OF SUBACUTE AND CHRONIC SUBDURAL HAEMATOMAS, EXAMINED WITHIN 24 HOURS AFTER LUMBAR PUNCTURE

Spectrophotometric pattern	Frequency (% of cases)	Xanthochromia (no. cases)		
		Visible	Visible?	Invisible
H	30.0	1	3	2
H+S, or S+H	60.0	5	1	6
S+H?	5.0		1	
S	5.0	1		

b. SPECTROPHOTOMETRIC SUBDURAL-HAEMATOMA FINDINGS IN 24 FLUIDS EXAMINED

Spectrophotometric pattern	Frequency (% of cases)
H	45.8
H+S, or S+H	45.8
S	8.3

For explanation of the symbols of the spectrophotometric patterns see Table 1.

For explanation of the symbols of the spectrophotometric patterns see Table 1. The patients are identified from Table 2. Tr. of haem. comp. = Traces of xanthochromic compounds of haemorrhagic origin.

FIG. 2. Spectrophotometric findings in electrophoretically separated protein fractions of CSF and corresponding subdural fluid. Spectrophotometric examinations performed with a Beckman DB-G spectrophotometer with scale expander. Wavelength in nm on the abscissa. A1. Original CSF. A2. Detail study of A1, scale expansion '5'. B. Albumin fraction of A1 with methaemalbumin, scale expansion '5'. C1. Beta-globulin fraction of A1 with oxyhaemoglobin and methaemoglobin compounds, scale expansion '5'. C2. Detail study of C1, scale expansion '10'. D1. Subdural fluid, diluted $\times 600$ with physiological NaCl. D2. Detail study of D1, scale expansion '5'. E1. Albumin fraction of D1 with methaemalbumin, scale expansion '5'. E2. Detail study of E1, scale expansion '10'. F1. Beta-globulin fraction of D1 with oxyhaemoglobin and methaemoglobin compounds. F2. Detail study of F1, scale expansion '5'.

globulin fractions in four cases obviously due to methaemoglobin and oxyhaemoglobin. Spectrophotometric examinations of the protein fractions were carried out in six of the 12 CSF samples separated electrophoretically. In accordance with previous investigations (Kjellin, 1969, 1971a) methaemalbumin and bilirubin compounds were found in the albumin fraction, and oxyhaemoglobin and methaemoglobin compounds in the CSF-beta₁-globulin region, methaemalbumin compounds being found most frequently (in all six cases examined). See also Fig. 2 (A-C).

The CSF-protein concentrations were within normal limits (≤ 50 mg/100 ml) in nine of the 25 cases with subdural haematomas, slightly or moderately increased (50–75 mg/100 ml) in seven cases, while a more pronounced increase (75–100 mg/100 ml) was found in eight cases, and more than 100 mg/100 ml in one case (316 mg/100 ml). The CSF-protein concentration was slightly increased in the single case with epidural haematoma.

The cell examinations showed a slight or moderate increase of predominantly monocytes in three of the 26 cases examined. Nothing noteworthy was found in the remaining 23 cases including five cases with traumatic lumbar puncture bleedings.

Twenty-four subdural fluids were examined from 23 patients, fluids from double-sided haematomas being examined in one case. The findings in CSF and subdural fluids are compared in Table 4. The spectrophotometric patterns found in each fluid can be compared in 20 of the 25 cases. Similar patterns were found in nine cases, and rather similar patterns (H+S/S+H) in two cases. In seven cases the H-component was in common for both fluids, and in one case the S-component, the spectrophotometric patterns of the two fluids diverging in regard to the other component (S or H). In the remaining case, H-pattern was observed in the subdural fluid but S-pattern (of 'old' appearance with only bilirubin) in the corresponding CSF. The spectrophotometric findings in the 24 subdural fluids examined, are summarized in Table 3b.

The protein fractions found upon electrophoresis of 14 subdural fluids were examined spectrophotometrically. Methaem-compounds

were detected in all cases, and oxyhaemoglobin in all but one. Methaemalbumin as well as methaemoglobin compounds appeared in all but two cases in which only methaemalbumin or methaemoglobin compounds were observed. Methaemalbumin dominated the xanthochromic pattern in two fluids, methaemalbumin and bilirubin compounds in two, methaemalbumin and methaemoglobin compounds in one, methaemoglobin compounds in five, methaemoglobin compounds and oxyhaemoglobin in one, and oxyhaemoglobin in three cases. See also Fig. 2 (D-F).

COMMENTS

The extremely high sensitivity of spectrophotometry in detecting xanthochromic compounds makes the method very suitable for examination of CSF in suspected vascular diseases of the central nervous system and meninges. In subacute and chronic subdural haematomas the CSF is often colourless or the visible xanthochromia is questionable as was also found in our cases (see Table 3a). However, xanthochromic compounds of haemorrhagic origin were found in all 25 cases with subacute and chronic subdural haematomas examined spectrophotometrically, while definite visible xanthochromia was observed in only 28% and the CSF was judged as colourless in 52% of those cases. Furthermore, characteristic bleeding patterns as given in Table 1 were detected in the CSF in 21 of the 25 cases. The remaining four cases had been spectrophotometrically examined four days and in one case three weeks after the lumbar punctures, and these delays probably caused the difficulties of classification. It is possible that they might have been classified if CSF volumes large enough for adequate ultrafiltration of the samples had been available, in accordance with one CSF, concentrated $\times 10$ and examined five days after lumbar puncture (see Table 2).

Haematoma patterns (with H-component) were found in the CSF in 90–95% of 20 cases with subdural haematomas, examined by CSF spectrophotometry within 24 hours after lumbar puncture. It is conceivable that methaem compounds might have been detected in the single case with spectrophotometric S-pattern in the

CSF if the xanthochromic compounds had been separated electrophoretically.

It must be especially emphasized that correction for traumatic lumbar puncture bleeding can generally be performed by collecting two or more consecutive CSF samples during lumbar puncture (see Hellström and Kjellin, 1971). In cases with subarachnoid haemorrhage and spectrophotometrically massive S-pattern overshadowing other CSF xanthochromic abnormalities, separation of the xanthochromic CSF compounds and/or repeated lumbar punctures generally solves this problem (Söderström and Kjellin, 1972).

Similar or rather similar spectrophotometric patterns were found in the CSF and the corresponding subdural fluid in 11 of 20 cases where the patterns of the both fluids could be compared. In seven of the remaining nine cases an H-component was in common for both fluids. In only one case the CSF and the corresponding subdural fluid had no spectrophotometric feature in common.

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REFERENCES

- Barrows, L. J., Hunter, F. T., and Banker, B. Q. (1955). The nature and clinical significance of pigments in the cerebrospinal fluid. *Brain*, **78**, 59–80.
- Hellström, B., and Kjellin, K. G. (1971). The diagnostic value of spectrophotometry of the CSF in the newborn period. *Developmental Medicine and Child Neurology*, **13**, 789–797.
- Kjellin, K. G. (1969). The binding of xanthochromic compounds in the cerebrospinal fluid. Preliminary report. *Journal of the Neurological Sciences*, **9**, 597–601.
- Kjellin, K. G. (1970). Xantokromi i likvor. (Abstract.) *Nordisk Medicin*, **83**, 705–706.
- Kjellin, K. G. (1971a). Xanthochromic protein compounds in the cerebrospinal fluid. In *Third International Meeting of the International Society for Neurochemistry, Budapest, 1971. Abstracts*, p. 175. Akadémiai Kiadó: Budapest.
- Kjellin, K. G. (1971b). Bilirubin compounds in the CSF. *Journal of the Neurological Sciences*, **13**, 161–173.
- Kjellin, K. G. (1971c). Xanthochromic protein compounds in the cerebrospinal fluid. (Abstract.) In *Proceedings of the Annual General Meeting of the Swedish Society of Medical Sciences*, p. 412. Svenska Läkaresällskapet: Stockholm.
- Kjellin, K. G. (1972). Isoelectric focusing of xanthochromic CSF compounds. (Abstract.) In *Proceedings of the Annual General Meeting of the Swedish Society of Medical Sciences*, p. 300. Svenska Läkaresällskapet: Stockholm.
- Kjellin, K. G. (1973). Isoelectric focusing of protein compounds in the cerebrospinal fluid. In *Fourth International Meeting of the International Society for Neurochemistry, Barcelona, 1973. Abstracts*, p. 167. International Congress Series No. 296. Excerpta Medica: Amsterdam.
- Kjellin, K. G., and Steiner, L. (1971). CSF spectrophotometry as diagnostic aid in chronic subdural haematomas.—A preliminary report. (Abstract.) In *Proceedings of the Annual General Meeting of the Swedish Society of Medical Sciences*, p. 413. Svenska Läkaresällskapet: Stockholm.
- Kjellin, K. G., and Söderström, C. E. (1973). Diagnostic significance of CSF spectrophotometry in cerebrovascular diseases. In *X International Congress of Neurology, Barcelona, 1973. Abstracts*, p. 167. International Congress Series No. 296. Excerpta Medica: Amsterdam.
- Kjellin, K. G., and Söderström, C. E. (1974). Diagnostic significance of CSF spectrophotometry in cerebrovascular diseases. *Journal of the Neurological Sciences*. (In press.)
- Kronholm, V., and Lintrup, J. (1960). Spectrophotometric investigations of the cerebrospinal fluid in the near-ultraviolet region. *Acta Psychiatrica et Neurologica Scandinavica*, **35**, 314–329.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275.
- Söderström, C. E., and Kjellin, K. G. (1972). Diagnostic significance of the CSF spectrophotometry in vascular neurological diseases. (Abstract.) In *Proceedings of the Annual General Meeting of the Swedish Society of Medical Sciences*, p. 301. Svenska Läkaresällskapet: Stockholm, 1972.