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Correlation between the post-mortem cell content of cerebrospinal fluid and time of death

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Summary. The number of cells in the cerebrospinal fluid increases after death. It is not clear whether this observation represents a post-mortem or a supravital phenomenon. There is speculation that some of the cells actively enter the cerebrospinal fluid during the first hours after death and that the rest exfoliate from the subarachnoid layer. The post-mortem cell count in cerebrospinal fluid correlates to the time after death and can be described mathematically (polynomial curve of third order).

Key words: Cerebrospinal fluid – Lumbar puncture – Post mortem – Pleocytosis – Time of death

Zusammenfassung. Die Zellzahl im Liquor cerebrospinalis steigt nach dem Todeseintritt an; es handelt sich aller Wahrscheinlichkeit nach um einen supravitalen oder postmortalen Prozeß. Unsere Beobachtungen sprechen dafür, daß ein Teil der Zellen in den ersten Stunden aktiv in den Liquorraum gelangt. Bei den restlichen Zellen dürfte es sich um abgeschilferte Zellen der weichen Hirnhaut handeln. Die postmortal bestimmte Zellzahl läßt sich mittels einer Formel einer Liegezeit zuordnen (Polinomialkurve dritter Ordnung).

Schlüsselwörter: Liquor – Lumbalpunktion – Postmortal – Pleozytose – Todeszeit

Introduction

Estimation of the time of death is one of the more difficult tasks in legal medicine. Unfortunately there is no accurate method to determine the exact time of death. The longer the post-mortem interval, the less precise is the medicolegal estimation. Most post-mortem changes are due to autolysis and heterolysis. Autolytical processes implicate the destruction of the chemical, physical and morphological organisation of a corpse without involvement of bacteria. Rectal temperature, post-mortem excitation of skeletal and non-skeletal (iris) muscles as well as physicochemical constants in body fluids can be measured. Attempts have been made to describe these post-mortem alterations in an objective way (Marshall and Hoare 1962; Mallach 1964; Green and Wright 1985; Henssge 1982, 1988, 1992; Henssge et al. 1988; Albrecht et al. 1990; Madea and Henssge 1990). It has also been proposed to introduce post-mortem laboratory diagnostics to determine physical and chemical shifts in corpses (Schleyer 1958; Coe 1974, 1977; Madea et al. 1987, 1989) in relation to the time of death.

All mortal signs which are routinely used to estimate the time of death may be influenced by a variety of internal and external factors. Therefore the interpretation of these signs is often influenced by subjective judgement.

During an investigation of post-mortem samples of cerebrospinal fluid (CSF) for other reasons, a marked pleocytosis was observed despite the lack of a history of neurological disorders or neuropathological changes. Preliminary studies showed that the number of cells in CSF increases during the post-mortem interval. To investigate the possible correlation of the number of cells and the post-mortem time interval, 3 series of corpses were investigated.

Material and methods

Lumbar punctures were performed on patients who died in hospital over a period of about 2 years. In 35 cases (hospital collective 1, shown in Table 1) the time of death was known. Only corpses with no clinical or autopsy evidence of lesions of the central nervous system were included in the study; an examination of the spinal cord was not routinely performed. The age of the deceased patients varied from 16 to 90 years old. The 35 corpses were placed dorsally at room temperature (ca. 20°C). Lumbar punctures were performed at different post-mortem intervals (3–39 h post-mortem). After rotating the stiff body onto the left side about 1 ml of CSF was collected with a spinal needle (Yale Spinal 19G3½ – 1.1 × 90 mm, Becton Dickinson) by puncturing the sub-

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Table 1. Time of death of hospital collective 1 (storing temperature 20°C, $n = 35$) and hospital collective 2 (storing temperature 4°C, $n = 34$) and corresponding postmortem CSF cell counts

Hospital collective 1 (20°C)			Hospital collective 2 (4°C)		
Case no.	Time after death	CSF cell count	Case no.	Time after death	CSF cell count
1	2.25	3	1	3	9
2	2.33	3	2	5.75	5
3	3	7	3	10.15	8
4	3	11	4	10.25	10
5	3.5	19	5	10.5	9
6	3.5	8	6	12.5	5
7	3.5	3	7	13	17
8	4	10	8	14.5	19
9	4.5	9	9	15.15	8
10	5	6	10	17.3	11
11	6.7	23	11	17.5	24
12	6.75	7	12	18	29
13	7	12	13	19.5	19
14	8.67	45	14	20	14
15	9	3	15	20.45	17
16	9	18	16	20.5	16
17	11	21	17	20.75	16
18	11.5	41	18	21.5	36
19	12.75	28	19	22	21
20	13	38	20	23	28
21	13.75	14	21	26.25	33
22	14.17	28	22	26.67	20
23	15.5	24	23	32.5	45
24	16	53	24	33.3	22
25	16	28	25	33.75	43
26	17	51	26	34.5	59
27	20.5	63	27	36.3	39
28	21	46	28	36.3	31
29	21.25	57	29	40	36
30	22	59	30	48	71
31	22	81	31	48.15	79
32	23	66	32	48.5	68
33	27.5	109	33	49.3	70
34	28.5	154	34	53.25	67
35	39	235			

arachnoid space at L2–3 or L3–4. In a few cases samples from the suboccipital subarachnoid space were also investigated. No problems are usually encountered when performing a lumbar puncture, however, the same difficulties arise as under clinic circumstances, i.e. adipositas, degenerative changes of the vertebral column and the possibility of damaging a blood vessel. If artificial bleeding has occurred CSF cannot be used for the determination of the post-mortem cell content. The cellular content of all samples was determined using the procedures of the Liquor Laboratory of the Neurological Clinic, University Hospital of Zurich. One part Samson reagent (acid. acet. liquefact, 30 ml, phenol 85% 2 ml, alcoholic Fuchsin 10% 2 ml, aqua dest ad 100 ml) and 10 parts CSF were aspirated into a haematological pipette (Assistent). After shaking the content of the pipette for about 2 min, 3 drops were discharged and the 4th drop was placed into a Fuchs-Rosenthal counting chamber which is divided into 256 squares. The cells were counted in the chamber by scanning all the squares. The number obtained corresponds to the cell count of 3 mm^3 CSF. In order to calculate the absolute cell count it is necessary to divide the number by three.

A 2nd group of 34 bodies (hospital collective 2, shown in Table 1) was examined in an analogous way. The bodies, however, were placed in a cold-storage chamber at 4°C immediately after death; in this group the post-mortem intervals of the lumbar punctures varied from 3 to 53 h.

The 3rd group comprised medico-legal cases (medico-legal collective) found at sites with temperatures of about 20°C (18–23°C). This group was investigated to test the usefulness of the cell count method in CSF to estimate the time of death. In all cases there were absolutely no anamnestic data of neurological disorders. In parallel to the cell count method, the time of death was also estimated using routine diagnostic parameters such as rigor mortis, livores, rectal temperature using a nomogram (Henssge 1982) and the idiomuscular bulge.

Results

A. Hospital Collectives (1 + 2)

Figure 1 shows the results of the cell count of postmortem CSF samples in the 2 hospital collectives stored at 20°C and 4°C in relation to the post-mortem period.

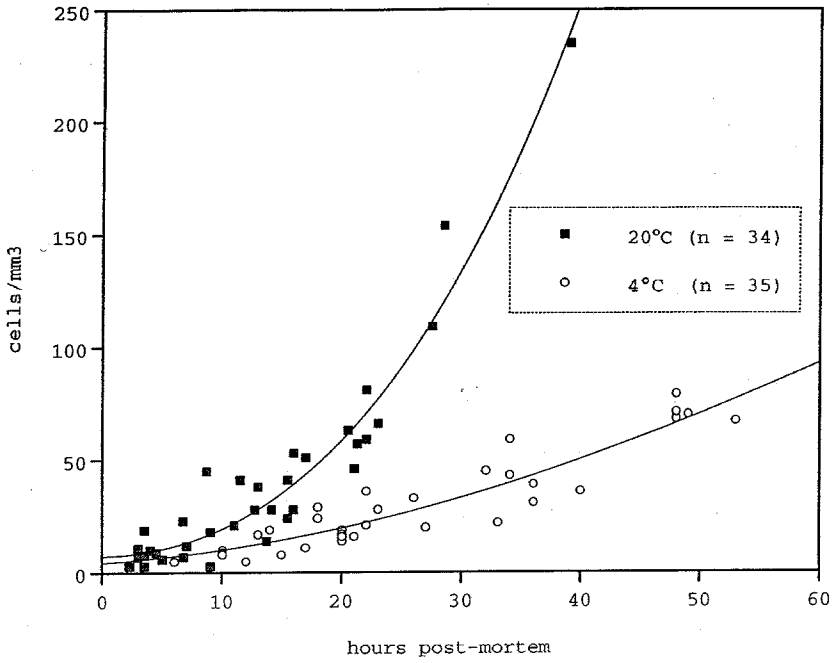

Fig. 1. Cell counts in post-mortem samples of lumbar cerebrospinal fluid (CSF): time of death fits best with a polynomial curve of third order. Results are shown for the bodies stored at 20° and 4°C. $y(4) = 0.00005x^3 + 0.022x^2 + 0.344x + 4.541$, $r = 0.927$; $y(20) = 0.001x^3 + 0.092x^2 + 0.137x + 7.408$, $r = 0.969$

The distribution of the values was fitted mathematically by a polynomial curve of the third order:

4°C $y = 4.541 + 0.344x + 0.022x^2 - 0.00005318x^3$ ($r = 0.927$)

20°C $y = 7.408 + 0.137x + 0.092x^2 + 0.001x^3$ ($r = 0.969$)

y: number of cells in CSF
x: hours post-mortem

The third order polynomial curves represent the course of the 'predicted values'. We used the computer software JMP 2.0.4 from Macintosh (Copyright SAS Institute) to evaluate and solve this equation to get the values 'hours post-mortem'. The mathematical basis is shown in Table 2.

To evaluate the apparent ascent of the polynomial curve beyond about 15 h post-mortem, groups of samples were formed at 5 hours intervals. It can thereby be demonstrated that cell counts in these groups are different for a post-mortem period greater than 10–15 h for the bodies stored at 20°C and at 4°C (Mann-Whitney test). Overall the groups are significantly different (Kruskal-Wallis test $P(20) = 0.0001$; $P(4) = 0.001$). The percentiles of the cell counts within these groups – marked for the 50th percentile – demonstrate the same trend as the third order polynomial curve shown. We are aware of the influence of the small number of values obtained from bodies stored for longer periods (> 25 hpm).

All the values represent transverse values. The longitudinal post-mortem changes of the cell count in lumbar CSF were evaluated with several punctions from the same body within intervals of a few hours. A marked increase in the number of cells counted was observed (Fig. 3). In the suboccipital samples of CSF only a few cells were noticed even hours after death.

B. Medico-Legal collective

In contrast to the hospital collectives (1 + 2) the time of death of the medico-legal cases was unknown. All the estimations of the time of death based on cells counts of post-mortem CSF using the relationship shown in Fig. 1 were within the range of estimations of time of death based on routine parameters. The results based on the cell counts showed a good correlation when compared to those values established with the nomogram of Henssge (Henssge 1982) (Fig. 4).

Discussion

Pleocytosis in the cerebrospinal fluid (CSF) is usually a diagnostic sign for the presence of a disease of the central nervous system. The arachnoid mater underlies the dura mater, sheet-like and filiform trabeculae traverse the subarachnoid space to joint the pia which covers the cerebral cortex and the spinal cord. Although several reports have established the ultra-structure of the human arachnoid mater, controversy still surrounds the nature of human pia mater (Alcolado et al. 1988). In post-mortem samples of lumbar CSF the cell count increases in relation to the length of the post-mortem period. Our investigations of post-mortem CSF samples allowed us to describe the increase in the number of cells by a mathematical function (polynomial curve of third order). Hence cell counts in postmortem CSF and time of death are correlated and can be used as a means to estimate the time of death.

Lumbar CSF normally contains only a few cells (2–4/mm^3) whereas ventricular and cysternal CSF is practically free of cells. Cytologically, 3 groups of cells can be differentiated into 'lymphocytoid', 'monocytoid' and 'reticular' cells (Dufresne 1973). Reticular cells may be polynucleated and lack cytoplasm. They appear shortly

Table 2. Solutions for a cubic equation (Gellert et al. 1966)

Cubic function	$Ax^3 + Bx^2 + Cx + D = 0$	
Normal form	$x^3 + rx^2 + sx + t = 0$	$r = \frac{B}{A}, \quad s = \frac{C}{A}, \quad t = \frac{D}{A}$
Reduced form	$y^3 + py + q = 0$	$p = s - \frac{r^2}{3}, \quad q = \frac{2r^3}{27} - \frac{rs}{3} + t$
Cardanic formula	$\left(\frac{q}{2}\right)^2 + \left(\frac{p}{3}\right)^3 \geq 0$	$u_1 = \sqrt[3]{-\frac{q}{2} + \sqrt{\left(\frac{q}{2}\right)^2 + \left(\frac{p}{3}\right)^3}}$
	One real solution and two conjugated complex solutions, which produce for	$v_1 = \sqrt[3]{-\frac{q}{2} + \sqrt{\left(\frac{q}{2}\right)^2 + \left(\frac{p}{3}\right)^3}}$
	$\left(\frac{q}{2}\right)^2 + \left(\frac{p}{3}\right)^3 = 0$	$y_2 = u_1 + v_1$
	One solution counting twice	$y_{2,3} = -\frac{u_1 + v_1}{2} \pm \frac{u_1 - v_1}{2} \cdot i\sqrt{3}$
Casus irreducibilis	$\left(\frac{q}{2}\right)^2 + \left(\frac{p}{3}\right)^3 < 0$	$r = \sqrt{\frac{-p^3}{27}}, \quad \cos\phi = -\frac{\frac{q}{2}}{\sqrt{\frac{-p^3}{27}}}$
	Three real solutions	$y_1 = 2\sqrt[3]{r}\cos\frac{\phi}{3}$ $y_2 = 2\sqrt[3]{r}\cos\left(\frac{\phi}{3} + 120°\right)$ $y_3 = 2\sqrt[3]{r}\cos\left(\frac{\phi}{3} + 240°\right)$

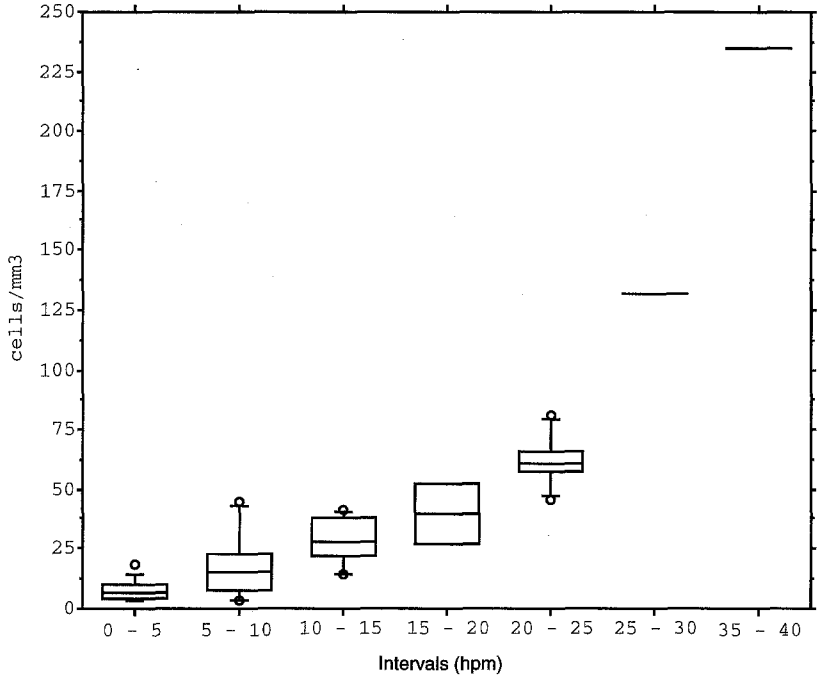

Fig. 2. Cell counts in post-mortem samples of CSF. Results are shown as box plots of groups formed at 5 hour intervals (20° C). The groups are significantly different (Kruskal-Wallis test P (20) = 0.0001). The percentiles of the cell counts within these groups – marked for the 50th percentile – demonstrate the same trend as the third order polynomial curve shown in Fig. 1

after an irritation of the central nervous system and are capable of phagocytosis. Based on immunohistochemical and immunocytochemical examinations they are probably of mesenchymal origin (Rutka et al. 1986a, b). In normal CSF astrocytes are usually absent (Trojanowski et al. 1986; Li et al. 1989). McGarry et al. (1969) stated that the origin of post-mortem CSF cells is either cells which have migrated from the blood (lymphocytes), the pia-arachnoid mesothelium or cells from the ependymal layer.

Platt et al. (1989) reported that CSF pleocytosis in post-mortem CSF is a common finding. Furthermore, he and his co-workers showed that immunocytochemically type-

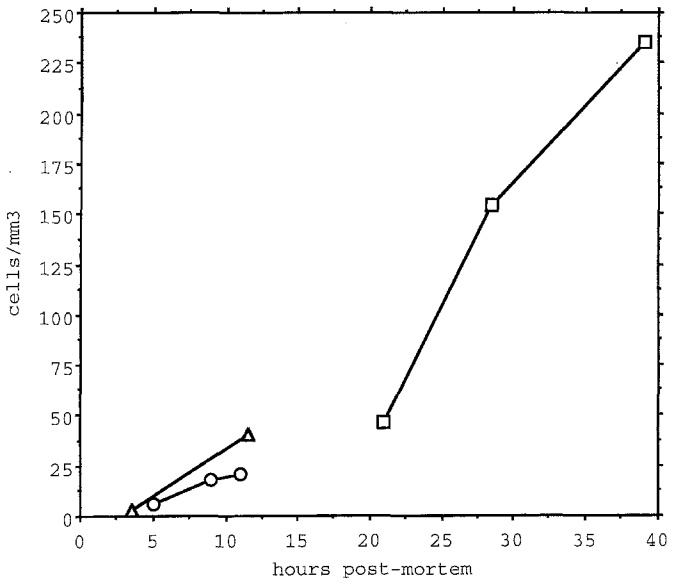

Fig. 3. Cell counts of CSF's in 3 corpses at different post-mortem interval (longitudinal evaluation). ○ = Body 1; □ = Body 2; △ = Body 3

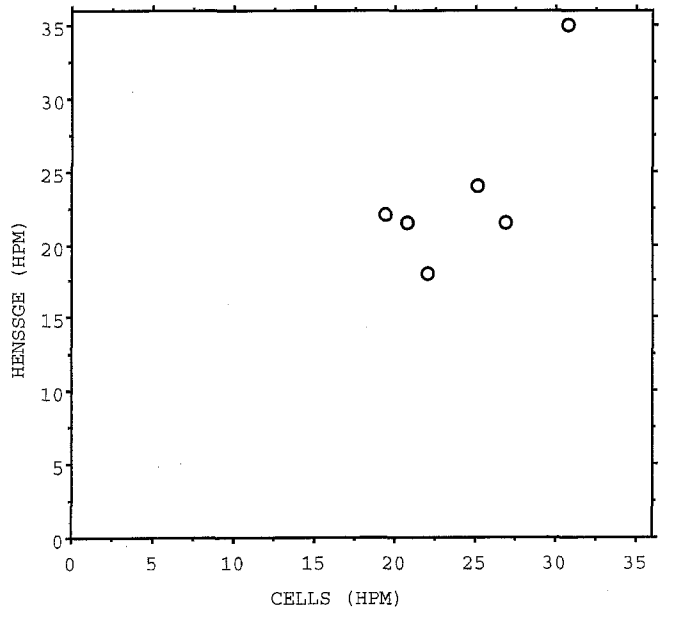

Fig. 4. Correlation of the time of death based on the cell count in CSF (20°C) and the body cooling temperature using the nomogram of Henssge

able cells were mononuclear and consisted of ca. 60–70% lymphocytes and ca. 20–40% macrophages. After a post-mortem period of more than ca. 12 hours the cells became vacuolated and (immuno-)histochemical identification became impossible.

In our study we tried to identify the cells in post-mortem CSF by cytochemistry (May-Grünwald, PAS), immunocytochemistry (Vimentin, Lysozym) and electron microscopy (data not shown). There is some evidence that a few cells possess phagocyte activity, because cytoplasmic incorporation of lipid-containing granules were observed. Immunocytochemically, a positive reaction with the antibody against Vimentin was noted. However, it is well known that cells of different origin suspended in fluids may express this intermediate filament (Ramaekers et al. 1984; Stosiek and Kaspar 1988; McGuire et al. 1989). Microscopically the cytoplasm of the observed cells is often swollen and vacuolated and we assume that these changes become more prominent in relation to the length of the post-mortem period. Around the 3rd (20°C) or 4th day (4°C) postmortem the cells tended to burst.

Sayk (1960) described a cranio-caudal increase of the cell count per mm^3 CSF in vital samples. We observed a high cell count in post-mortem lumbar CSF and only a few cells in the suboccipital CSF. As an explanation we assume a post-mortem sedimentation of exfoliated arachnoid and pia cells. The presence of cells with phagocytic activity in post-mortem CSF may indicate a supravital invasion of macrophages.

Our first results suggest a correlation between cell count in post-mortem CSF and post-mortem interval. Subsequent studies will be necessary to investigate the influence of the surrounding temperature, the position of the body and other external factors. Another goal is to establish the confidence intervals of the estimations.

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