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Postmortem biochemistry performed on vitreous humor after postmortem CT-angiography

Silke Grabherr [a,1], Christèle Widmer [a], Katia Iglesias [b], Frank Sporkert [a], Marc Augsburger [a], Patrice Mangin [a], Cristian Palmiere [a,*,1]

[a] University Center of Legal Medicine, Lausanne–Geneva, Rue du Bugnon 21, 1011 Lausanne, Switzerland
[b] Institute of Social and Preventive Medicine, Lausanne University Hospital, Rue du Bugnon 17, 1004 Lausanne, Switzerland

A B S T R A C T
Postmortem angiography is becoming increasingly essential in forensic pathology as an adjunct to con-ventional autopsy. Despite the numerous advantages of this technique, some questions have been raised regarding the influence of the contrast agent injected on the results of toxicological and biochemical anal-yses. The aim of this study was to investigate the effect of the injection of the contrast agent Angiofil®, mixed with paraffin oil, on the results of postmortem biochemical investigations performed on vitreous humor. Postmortem biochemical investigations were performed on vitreous samples collected from bodies that had undergone postmortem angiography (n = 50) and from a control group (n = 50). Two vit-reous samples were analyzed for each group and the results compared. Glucose, urea, creatinine, 3-β-hydroxybutyrate, sodium and chloride were tested. Different values were observed between the first and second samples in each group. However, these differences were not clinically relevant, suggesting that the injection of this contrast agent mixture does not modify the concentration of the analyzed sub-stances in the vitreous humor.

Keywords:
Postmortem biochemistry; Glucose; Vitreous humor; Postmortem angiography; Forensic radiology

1. Introduction

Postmortem angiography is becoming increasingly prominent in forensic pathology as an adjunct to conventional autopsy [1–3]. Despite its numerous advantages, forensic pathologists and toxicologists have questioned certain elements of this technique. These questions pertain primarily to the influence that the injection of contrast agent mixtures may have on toxicological and biochemical analyses [2]. Consequently, it has been proposed that samples for toxicology and postmortem biochemistry be collected prior to the injection of contrast agents into the vascular system. While several studies have investigated the application of postmortem angiography in forensic pathology, no studies have yet reported the effects of contrast agent injection on postmortem biochemical investigations of the vitreous humor.

Postmortem computed tomography angiography (PMCTA) is routinely performed at the University Centre of Legal Medicine, Lausanne–Geneva as an integral part of the forensic autopsy process in all traumatic deaths and in all cases of sudden unexpected death. The bodies are admitted to the medico-legal center by local investigative authorities to ascertain the cause of death and, depending on the circumstances, determine the sequence of events that led to death. Systematic analyses of vitreous glucose, sodium, chloride, urea nitrogen and creatinine are performed in all cases of sudden death. Additionally, in selected cases, levels of glycated hemoglobin, blood or vitreous 3-β-hydroxybutyrate, acetone and isopropyl alcohol as well as markers of inflammation, sepsis, anaphylaxis and cardiac ischemia are analyzed in postmortem serum obtained from femoral blood.

The aim of this study was to investigate whether or not and to what extent the injection of the contrast agent Angiofil® (mixed with paraffin oil) [2] affected postmortem biochemical investigations of vitreous humor and, consequently, to determine the necessity or utility of collecting vitreous samples before contrast agent injection. We focused on detecting glucose, urea nitrogen, creatinine, 3-β-hydroxybutyrate (3HB), sodium and chloride levels. Based on the recommendations of Karlovsek [4,5] and Zilg et al. [6] as well as our own experiences [7], antemortem hyperglycemia at our center is estimated based on vitreous glucose levels alone. Therefore, we considered neither vitreous lactate nor potassium levels in this study, as these measurements are not routinely performed at our facility.

* Corresponding author. Tel.: +41 021 314 49 61, mobile: + 41 79 556 69 89; fax: +41 021 341 70 90.
E-mail address: cristian.palmiere@chuv.ch (C. Palmiere).
[1] These authors are equally contributed as first authors to this work.

2. Materials and methods

2.1. Study design

The aim of this study was to investigate whether the injection of the contrast agent Angiofil® (mixed with paraffin oil) would modify the composition of the vitreous humor and, consequently, influence the results of postmortem biochemical investigations. To this end, we compared the results of postmortem biochemical investigations of two groups of forensic autopsy cases, a postmortem angiography group and a control group. For the angiography group, we collected an initial vitreous humor sample before performing PMCTA and a second vitreous sample afterwards. Approximately 6 h elapsed between these samplings, corresponding with the time necessary to perform the native CT-scan, the external examination of the body and the postmortem angiography. For the control group, we collected two vitreous samples approximately 6 h apart, as in the experimental group but without performing the angiography.

The main technical question concerned the collection procedure, for which two possibilities were considered. Our first option was to collect and pool aliquots of vitreous humor from both eyes, both before and after the angiography (or, for the control group, before and after 6 h). Our second option was to collect the first sample from one eye and the second sample from the other eye. We found the second option to be more technically practical.

To test the reliability of this approach and reduce the risk of misinterpretation of the results, we performed a preliminary study on 50 bodies which had not undergone postmortem angiography and compared the results of biochemical investigations of vitreous humor obtained from the left and right eye.

2.2. Preliminary study

The aim of this preliminary study was to compare the results of biochemical investigations of the vitreous humor obtained from the left eye with those obtained from the right eye. The study was performed on 50 forensic cases (25 males and 25 females) with various causes of death, including hanging, myocardial infarction, subarachnoid hemorrhage, and death following heroin, methadone, and cocaine consumption. Glucose, urea nitrogen, creatinine, sodium and chloride levels were determined. Severely decomposed bodies and bodies with severe cranial destruction were excluded from the study as were bodies suspected of carrying primary ocular diseases or ocular traumas.

The study was carried out as follows: samples of vitreous humor were separately collected from the left and right eyes as soon as possible upon arrival of the body at the morgue (usually 1–12 h after death). Vitreous samples were collected by aspiration using a sterile needle and a syringe through a scleral puncture at the lateral canthus. After collection, the samples were immediately centrifuged at 3000*g* for 15 min. The separated supernatant was collected, stored in tubes without preservatives, frozen at −20 °C and used for analysis. No specimens were excluded because of insufficient sample volume. Sodium, chloride, urea nitrogen, creatinine and glucose were determined on a Dimension® Xpand® Plus Integrated Chemistry System (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). All concentrations were determined without diluting samples.

2.3. Main study

2.3.1. Subjects

One hundred cases were included in the main study (50 males and 50 females). The angiography group (PMCTA group) and the control group were both made up of 50 cases, 25 males and 25 females. The mean age was 63.2 years in the PMCTA group and 61.5 years in the control group. The postmortem interval as well as the cause and the circumstances of death were the criteria for inclusion in the study. Samples from severely decomposed bodies and from bodies with severe cranial destruction were excluded as were bodies suspected of carrying primary ocular diseases or ocular traumas. The angiography group included forensic cases with medical records, personal histories and circumstances of death as well as cases in which native CT-scans suggested one of the following: cardiac death, cerebral or subarachnoid hemorrhages, thoracic and abdominal traumas involving the vascular system, or external or internal hemorrhages following stab wounds. The control group included forensic cases with various causes of death including hanging, manual strangulation, carbon monoxide intoxication, gunshot wounds, and death following heroin and methadone consumption. Bodies of both groups were supplied by forensic teams and deaths occurred outside hospital. Cardiopulmonary resuscitation was attempted in 37 cases out of 100 (23 cases in the PMCTA group and 14 cases in the control group). Neither glucose nor sodium bicarbonate was administered in any incident of cardiopulmonary resuscitation. Antemortem blood biochemistry results shortly before death were not available for any case.

2.3.2. Radiological investigations

All bodies included in this study underwent native CT-scans performed on an 8-row CT-unit (CT LightSpeed 8, GE Healthcare, Milwaukee, WI, USA) using the following scan parameters: field of view (FOV): 50 cm; slice thickness: 2.5 mm; reconstruction interval: 2 mm, 120 kVp and 280 mA; and scan time 150 s. The bodies of the PMCTA group additionally underwent postmortem angiographies which were performed using multi-phase postmortem computed tomography angiography following the standardized protocol of Grabherr et al. [2]. According to this protocol, all bodies were examined using the same quantity of contrast-agent mixture and the same perfusion parameters. To perform the angiography, cannulation of the femoral vessels on one side was performed using cannulas (MAQUET Gmbh & Co. KG, Rastatt, Germany) with a 16-French diameter for arteries and 18-French for veins. A recently developed pressure-controlled perfusion device (Virtangio®, Fumedica AG, Muri, Switzerland) was used to inject a mixture of contrast agent (Angiofil®, Fumedica AG, Muri, Switzerland) with paraffin oil (paraffinum liquidum, obtained at a local pharmacy). The total amount of injected liquid per body was 3300 ml including 6% of contrast agent (198 ml of Angiofil®). The arterial phase of PMCTA was carried out after the injection of 1200 ml (flow rate: 800 ml/min) of this mixture into one femoral artery using the following scan parameters: field of view: 50 cm; reconstructed slice thickness: 1.25 mm; reconstruction interval: 0.6 mm, 120 kVp and 280 mA; and scan time: 140 s. The venous phase of the angiography was performed post injection of 1600 ml (flow rate: 800 ml/min) of contrast-agent mixture into one femoral vein using the following scan parameters: field of view: 50 cm; reconstructed slice thickness: 2.5 mm; reconstruction interval: 1.2 mm, 120 kVp and 280 mA; and scan time: 140 s. Lastly, the so-called dynamic phase was performed in which an additional 500 ml of contrast-agent mixture was injected into the femoral artery over 2.5 min (flow rate 200 ml/min). Data acquisition using the same scan parameters as for the venous phase was performed during body perfusion.

2.3.3. Conventional autopsy

For both groups, the autopsies were performed immediately after the second sampling and within 48 h after death.

2.3.4. Sample collection

For all cases, an initial sample of vitreous humor was collected from the left eye as soon as possible upon arrival of the body at the morgue (usually 1–12 h after death). Bodies were stored at 4 °C before the collection of the first vitreous sample.

For the PMCTA group, the second vitreous sample was collected from the right eye approximately 6 h after the collection of the first sample. Between the first and the second samplings, the bodies of the PMCTA group underwent postmortem angiography with injection of the contrast agent in a specially equipped room at standard conditions of temperature (between 20 °C and 22 °C).

For the control group, the second vitreous sample was collected from the right eye approximately 6 h after the collection of the first sample. Between the first and the second sampling, the bodies were stored in an unrefrigerated mortuary at standard conditions of temperature (21 °C).

All vitreous samples were collected by aspiration using a sterile needle and a syringe through a scleral puncture at the lateral canthus. After collection, the samples were immediately centrifuged at 3000*g* for 15 min. The separated supernatant was collected, stored in tubes without preservatives, frozen at −20 °C and used for analysis. No specimens were excluded because of insufficient sample volume.

2.3.5. Sample analysis

Sodium, chloride, urea nitrogen, creatinine and glucose concentrations were determined on a Dimension® Xpand® Plus Integrated Chemistry System (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). The concentrations of all substances were determined without diluting samples.

3HB concentrations were determined by an enzymatic photometric method. Frozen samples of vitreous humor were thawed overnight at 4 °C and deproteinized with perchloric acid. Sample supernatants were used for analysis following centrifugation.

2.4. Ethical issues

Ethical matters were discussed with the local ethics committee and this study was authorized as a part of an investigation into medico-legal autopsies ordered by the judicial authorities.

2.5. Statistical method

Paired *t*-tests were conducted using Stata 11 software (Table 1) to analyze differences between left and right eyes samples, including differences in sodium, chloride, urea nitrogen, creatinine and glucose levels (preliminary study). Power analyses with an alpha of 5% for one sample equivalence test were computed according to Chow et al. [8].

Table 1
Preliminary study: comparison of analyte concentrations in right and left eye (50 subjects).

	Mean left eye (SD)	Mean right eye (SD)	Mean difference (SD)	*p*-Value
Sodium (mmol/l)	139.44 (6.54)	139.60 (6.04)	0.16 (0.98)	0.252
Chloride (mmol/l)	130.22 (8.51)	130.02 (8.48)	0.20 (1.11)	0.207
Glucose (mmol/l)	1.588 (0.663)	1.592 (0.651)	0.004 (0.081)	0.728
Urea nitrogen (mmol/l)	5.236 (1.734)	5.244 (1.712)	0.008 (0.101)	0.577
Creatinine (μmol/l)	84.84 (31.56)	84.96 (31.39)	0.12 (1.06)	0.428

Descriptive analyses (mean ± SD, median, min, max) of data from the main study are shown for each group, each time measurement and for the whole dataset underwent (Table 2 and Fig. 1).

ANOVAs with repeated measures (time as within factor and group as between factor) were performed with the Stata 11 (Table 3) to analyze the effect of PMCTA on sodium, chloride, urea nitrogen, creatinine, glucose and 3HB measurements.

3. Results

3.1. Preliminary study

Our preliminary study showed that concentration differences between left and right eye were not statistically significant (Table 1). This is in agreement with results previously reported by Mulla et al. [9] and Thierauf et al. [10].

To ensure our finding was not due to a lack of statistical power, we define a difference of 1 mmol/l for sodium and chloride, 1 μmol/l for creatinine and 0.1 mmol/l for urea and glucose as not clinically significant, and we found a power greater than 99.9% for a sample of 50 subjects in a test of equivalence for each analyte concentration.

3.2. Main study

Between the first and second measurements (Table 2), sodium, chloride, urea nitrogen, creatinine and 3HB increased and glucose decreased independently of the group. ANOVA tests ($p < 0.001$) demonstrated that the time effect was statistically significant for each analyte concentration tested.

To analyze the effect of postmortem angiography on sodium, chloride, urea nitrogen, creatinine, glucose and 3HB levels, the interaction between times and groups (Table 3) needed to be examined. The first and the second measurements were highly correlated (Fig. 1) and the differences between the two measurements were modest. Consequently, these differences were statistically but not clinically significant. To evaluate the interaction effect, we determined the effect size, the partial eta squared, which describes the proportion of total variation that may be attributed to a specific factor. In our study, these effects were slight.

4. Discussion

In the study herein presented, we compared postmortem biochemical analyses from two groups of forensic autopsies. Although the initial articles describe that the performance of PMCTA using the mixture of Angiofil® and paraffin oil does not hamper or hinder the performance of conventional autopsy and histology [2,11], its influence on postmortem biochemical analyses had not been investigated up to now. The first group underwent postmortem angiography using a contrast agent mixture of paraffin oil and Angiofil®. The second did not. We studied the effects of this treatment on our ability to measure the concentration of six analytes (sodium, chloride, glucose, urea nitrogen, creatinine and 3HB) that are commonly assayed in vitreous humor in our medico-legal center.

Our results with the six tested analytes indicate that different concentrations may be observed depending on the time of analysis in both studied groups. However, these differences are statistically but not clinically significant.

These observations suggest that the injection of this specific contrast agent mixture affects neither the composition nor the measurements of these substances in the vitreous humor. Consequently, vitreous humor sampled after the injection of the contrast

Table 2
Main study: comparison of the analyte concentrations (50 subjects by groups).

	Control group			PMCTA group		
	First measurement mean (SD)	Second measurement mean (SD)	Mean difference (SD)	First measurement mean (SD)	First measurement mean (SD)	Mean difference (SD)
Sodium (mmol/l)	139.94 (6.67)	141.58 (6.64)	−1.64 (.8514094)	137.76 (5.00)	139.38 (4.87)	−1.62 (.5674864)
Chloride (mmol/l)	119.76 (4.93)	121.48 (4.88)	−1.72 (.4965185)	118.78 (3.48)	120.30 (3.44)	−1.52 (.6141196)
Glucose (mmol/l)	1.70 (0.59)	1.51 (0.57)	.188 (.1533836)	1.68 (0.58)	1.50 (0.56)	.182 (.1507668)
Urea nitrogen (mmol/l)	5.52 (1.75)	5.70 (1.75)	−.184 (.0650275)	5.488 (1.81)	5.64 (1.79)	−.1559999 (.0501427)
Creatinine (μmol/l)	83.30 (14.10)	85.72 (14.30)	−2.42 (3.854973)	81.94 (19.47)	83.84 (19.49)	−1.9 (.6468132)
3HB (μmol/l)	161.68 (27.58)	163.36 (27.82)	−1.68 (.7938539)	163.66 (46.46)	164.52 (46.01)	−.86 (1.840696)

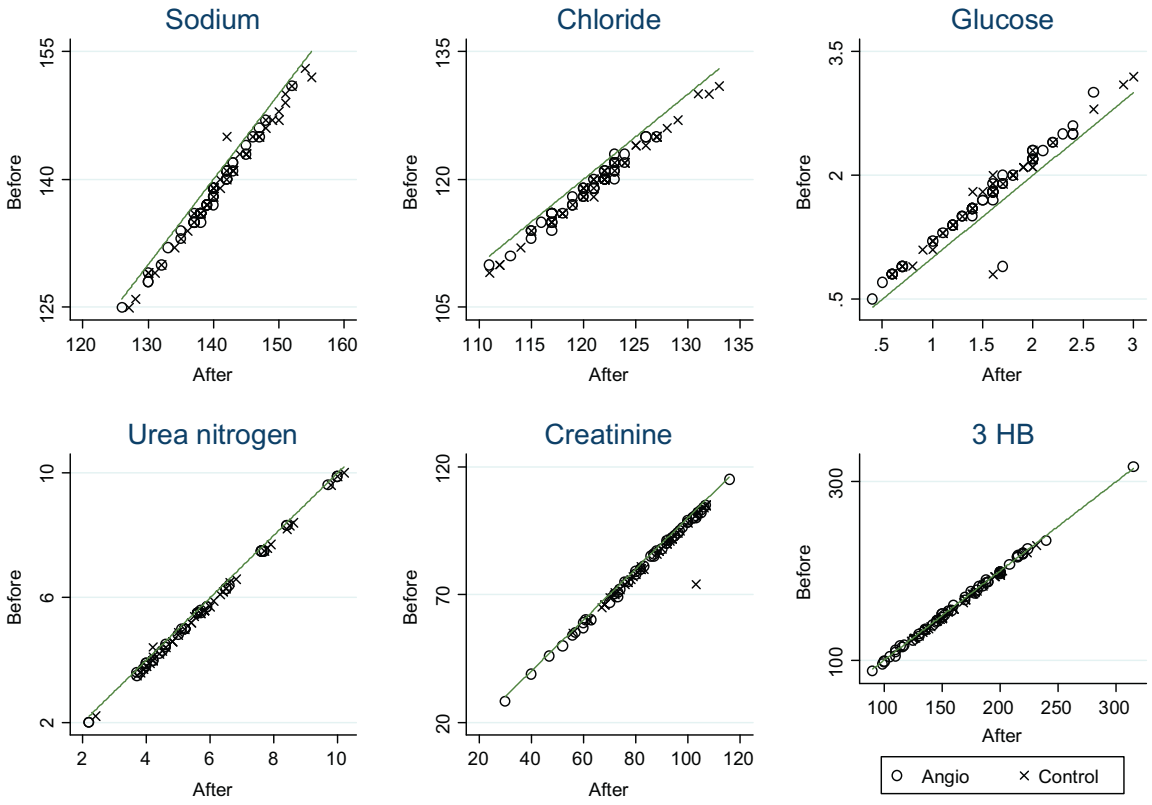

Fig. 1. Distribution of analyte concentrations by group and time measured (50 subjects per groups). The line corresponds to a perfect correspondence between measurements.

agent should be reliable for use in postmortem biochemical investigations.

One of the first reports concerning forensic biochemistry was published in 1940 and focused on postmortem blood glucose determination [12]. In 1959, Naumann [13] published the first report on the chemical constituents of human vitreous humor. Before then, the understanding of these constituents was limited to animals and enucleated human eyes. Subsequently, numerous researchers [4–7,9,10,14–33] have performed postmortem biochemical analyses on vitreous humor. These analyses typically focused on levels of vitreous glucose, lactate, sodium, chloride, potassium, urea nitrogen and creatinine. Determinations of vitreous 3HB have been also carried out and shown to correlate with the levels of 3HB in blood [31–33].

Coe [24] demonstrated a consistent decrease in vitreous glucose levels following death in non-diabetic individuals. Such reductions were observed as quickly as 4 or 5 h post death and vitreous glucose levels often approached zero. Conversely, he described slight effects of postmortem glycolysis on the elevated vitreous glucose

Table 3
Results of the analysis of the analyte concentrations: the effect of interaction time * group on the analyte concentrations.

	Interaction time * group		Effect size
	$F(1,98)$	p-Value	Partial eta squared
Sodium	0.02	0.890	0.0002
Chloride	3.21	0.076	0.0317
Glucose	0.04	0.844	0.0004
Urea nitrogen	5.81	0.018	0.0560
Creatinine	0.88	0.349	0.0089
3HB	8.37	0.005	0.0787

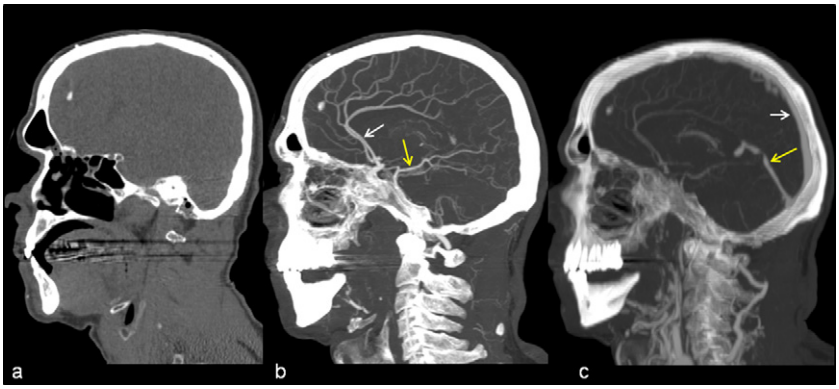

Fig. 2. Sagittal Maximum Intensity Projection (MIP) reformation of the head and the neck reconstructed from data obtained during native MDCT (a), arterial phase (b) and venous phase (c) of angiography. (a) In the native MDCT no vessels are visible. (b) The arterial phase of angiography is demonstrating the anterior (white arrow) and posterior (yellow arrow) cerebral circulation. (c) Venous phase is showing venous sinuses (white arrow: superior longitudinal sinus, yellow arrow: sinus rectus). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

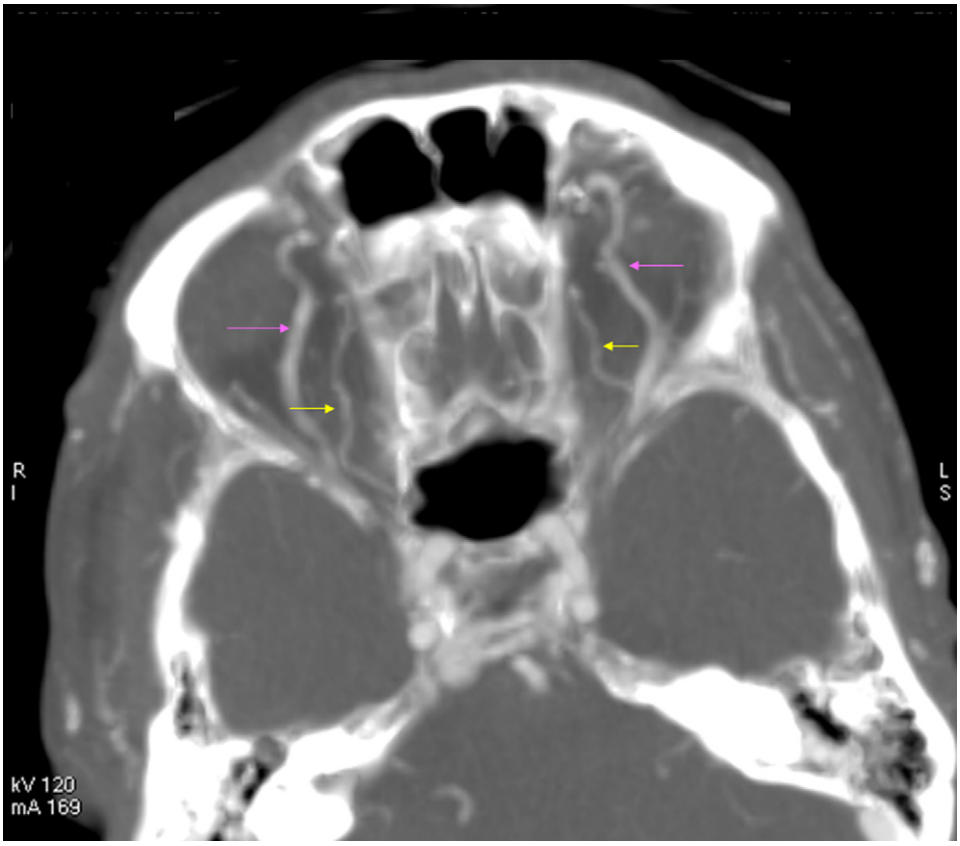

Fig. 3. Axial Maximum Intensity Projection reformation of the anterior cranial fossa reconstructed from data of the dynamic phase showing the opacified orbital vessels (yellow arrow: ophtalmic artery; pink arrow: superior ophthalmic vein). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

levels characterizing uncontrolled diabetic individuals, allowing cases of hyperglycemia to be easily diagnosed, even in embalmed bodies [34]. More recently, other authors have confirmed that vitreous glucose concentration is the most reliable marker for estimating antemortem hyperglycemia [4–7].

Vitreous sodium levels have been determined to be relatively stable during the early postmortem period. Sodium concentrations are similar to those found in the serum of living individuals. According to several authors, abnormalities in antemortem sodium concentrations in serum are reflected in postmortem vitreous levels, making it possible to diagnose hypo- or hypernatremia at the time of death. Postmortem vitreous chloride levels track with sodium, suggesting that abnormalities in antemortem serum chloride may be demonstrated with postmortem vitreous analyses, allowing diagnosis of electrolyte disturbances present at the time of death [9,10,24,29,35,36].

However, Madea and Lachenmeier [37] reported cases of increased vitreous sodium values that were not related to electrolyte disturbances and concluded that vitreous sodium and chloride values were insufficient metrics for determining the cause of death.

Leahy and Farber [14] found vitreous urea nitrogen to be in the normal range for individuals dying suddenly or with known nor-

mal antemortem blood urea nitrogen. Coe [24] observed vitreous urea nitrogen to be in the normal range for normal individual and to correspond with blood urea nitrogen over all ranges of urea. Moreover, elevated urea nitrogen concentrations have been found to remain high even in embalmed specimens [34]. Postmortem vitreous creatinine levels have been found to reflect blood levels, although being slightly lower than serum levels [24].

The study presented here is the first comparison between postmortem biochemical analyses of vitreous humor before and after the injection of the contrast agent used for the postmortem angiography. Previous studies have been limited to postmortem chemistries on vitreous humor before and after embalming [34,38,39].

No studies have yet been published documenting the effects of contrast agent mixtures used for postmortem angiographies on the outcome of postmortem biochemical investigations. Therefore, as of recent, no contrast agent could be injected without risking alteration of such analyses. To our knowledge, no test after the injection of other contrast agents has been performed. This may be due to the fact that postmortem angiography is not yet routinely used in the forensic pathology practice. Furthermore numerous medico-legal centers exclude postmortem biochemical analyses from routine postmortem investigations. Nevertheless, because postmortem angiography is increasingly employed in routine forensic pathology, it is necessary to investigate the influence of contrast agent mixtures on toxicological and biochemical analyses.

Chemically, the contrast agent Angiofil® is a mixture of esters (mainly ethyl esters) of polyiodinated fatty acids. Hence, it is an oily liquid showing all the chemical properties of such liquids. It is yellowish, nearly odourless and stable under normal conditions (room temperature). The advantages of using oily liquids for the injection into vessels are well known and have been described in a previous article [40] that stated that combining an oily perfusate with a lipophilic contrast agent allows postmortem circulation to be established and the performance of subsequent high-resolution angiography to be carried out. Microscopic studies reported in the same article revealed that the oil may block the capillary region due to fatty embolism, which is especially vulnerable to postmortem permeability. However, the oil enters the venous system by passing through small arteriovenous shunts while the capillary microcirculation is arrested. Thus, oily perfusates appear to be highly suitable for postmortem angiography. The same mechanism is used in cancer treatment to separate tumors from their blood supply during chemoembolization [41]. The level of this microembolisation depends on the viscosity of the oily perfusion. The use of an adequate perfusion liquid is therefore of utmost importance. If the contrast agent Angiofil® is dissolved in paraffin oil, its viscosity will be the same as that of the oil used. In order to perfuse a human body, paraffinum liquidum has proven to be the most appropriate [2]. The use of the more viscous paraffinum perliquidum however, may lead to significant extravasations, especially in regions with high autolytic activities such as the pancreas and gastric mucosa [40,42]. By diluting Angiofil® with a solvent such as decane, its viscosity can be decreased so as to enter the capillar-ies and become a contrast agent used in microangiography [43]. B y using Angiofil® together with paraffinum liquidum as solvent, as proposed according to the standardized protocol of PMCTA, the kinematic viscosity of the mixture is around 67–74 mm^2/s (measured at 40 °C). In this mixture, the perfusion of vessels visible in multidetector computed tomography (MDCT) (down to a diameter of 0.5 cm) is possible. PMCTA therefore allows the vascular system of the head to be visualized perfectly (Fig. 2). Furthermore, the vessels of the eye are perfused and main vessels can be easily identified on the obtained radiological images (Fig. 3). For this reason, we wanted to find out whether or not there is a risk to introduce the perfusion mixture into the vitreous humor and thereby create artifacts during the analysis of biochemical markers. Though the above proposed contrast agent mixture was noted as staying intravascular and therefore not appearing in the vitreous humor where it could influence biological markers, our aim was to prove this theory with our study.

Our results indicate that when bodies are stored at 4 °C before the collection of the first vitreous sample, postmortem angiography is performed in a specially equipped room at standard conditions of temperature (between 20 °C and 22 °C) and the second vitreous sample is collected approximately 6 h after the first collection, the injection of this specific contrast agent does not modify the vitreous composition. Consequently, the sampling for vitreous postmortem biochemistry analyses could theoretically be delayed until after angiography.

However, it is known that the concentration of several substances vary in the postmortem period. Such variance depends upon body storage conditions, the postmortem interval, the collected sample and the tested analyte.

Consequently, in our opinion, sampling for postmortem biochemical analyses should be not delayed but carried out as soon as possible following the arrival of the body at the morgue. Quick action will prevent changes from occurring in analyte concentrations and improve the reliability of postmortem biochemical findings.

Conflict of interest

The authors have no conflict of interest to declare.

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