Histological examination of carotid artery tissue in cases of ligature strangulation and hanging

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ABSTRACT
Violence against the neck can result in a range of macromorphological and micromorphological findings. However, the forensic relevance of the carotid sinus in cases of violence against the neck remains controversial. In this follow-up study of 22 cases of suicidal and accidental strangulations, carotid bifurcations were examined histologically for morphological changes implying direct trauma, including haemorrhage and immunohistochemical expression of heat-shock proteins 27, 60, and 70 and aquaporin-3. These cases were compared with a control group (82 cases) without neck compression or head trauma and with variable causes of death. No relevant histopathological findings implying direct trauma of the carotid bifurcation were found. No cases showed positive aquaporin-3 staining and only five cases showed positive heat-shock protein-27 staining, all of which were hangings. Without massive trauma of the carotid bifurcation, histological alterations cannot be expected. Without signs of rapid death, findings of acute circulatory failure, macromorphological and micromorphological findings of neck compression, and reliable markers indicating relevant impact on the carotid bifurcation the diagnosis of a lethal reflex cannot be verified.

KEY POINTS
• Among 22 cases of strangulation causing death, there were 16 cases of hanging and six cases of ligature strangulation.
• Few cases showed small haemorrhages located predominantly in the surrounding fat and connective tissues; however, the haemorrhages did not have any effects on the tissues.
• Neck compression had minimal effects on heat-shock protein-27 expression in carotid artery tissue.
• Aquaporin-3 staining suggested it is not a useful marker for relevant neck pressure, or there had not been any relevant neck impact.
• Our findings suggested no direct evidence for reflex cardiac death resulting from a brief force against the neck.

Introduction
The carotid bifurcation is involved in the regulation of blood circulation because of the presence of the carotid body and sinus. The carotid body is located within the bifurcation, between the internal and external carotid arteries, while the carotid sinus is located inside the vessel wall of the internal artery. Stimulation of either structure (e.g. by hypoxia or vasoconstriction) can induce changes in heart rate and blood pressure via chemoreceptor and pressoreceptor activity [1, 2]. The carotid sinus can also be stimulated by external pressure, leading to vessel wall deformation. The faster and more extensive the stimulation, the more extensive the inhibition of afferent sympathetic fibres and the stimulation of parasympathetic fibres, resulting in hypotension and bradycardia [3]. This effect is used clinically (termed the carotid sinus massage) to interrupt tachyarrhythmias, which rarely result in asystole [4, 5]. This reflex predominantly causes cardioinhibitory and vasodepressive effects [6]. Therefore, stimulation, particularly of the carotid sinus, can induce bradycardia up to asystole with circulatory failure [7–9]. In young and healthy subjects, unilateral pressure typically only causes a minor decrease in heart rate and blood pressure [10], although induced ventricular fibrillation has been reported in a few cases [11–13]. Most forensic pathologists believe that this cardioinhibitory reflex can, at least in theory, cause death [14].

In 1927, the German physiologist Heinrich Ewald Hering theorised that the carotid sinus may be affected in cases of violence against the neck. For example, in cases of hanging, a carotid sinus reflex...
with decrease in heart rate and blood pressure and rapid loss of consciousness may occur. However, in people with arteriosclerosis, light pressure to the neck is sufficient to induce unconsciousness. Nevertheless, his studies were predominantly performed on animals [15]. Therefore, the mechanisms by which the cardioinhibitory reflex occurs and whether evidence of a lethal reflex can be verified during human autopsy remains unknown. Furthermore, whether a minor neck trauma such as a short grip to the neck can cause sudden loss of consciousness and even death because of a carotid sinus reflex is unclear.

Herein, we performed a follow-up study of cases of death due to ligature strangulation and hanging. The study aimed to assess histological macroscopic findings (particularly haemorrhage in tissues surrounding the carotid bifurcations, the sinus, and the carotid body as potential direct indicators of tissue trauma and a cardiac reflex and expression of heat-shock proteins (Hsp) 27, 60, and 70 and aquaporin-3 (AQP-3) [16]), as well as the patients’ history.

Materials and methods

The study group consisted of 22 cases of ligature strangulation and hanging, in which autopsy was performed at our Institute of Legal Medicine. All available information was obtained from autopsy protocols or documentations by investigation authorities. The study group comprised seven female and 15 male individuals, with a mean age of 54.04 years (range, 10–91 years). The control group comprised 82 cases with natural and nonnatural causes of death, including myocardial infarction or head shot wounds, with different agonal periods but without evidence of neck trauma [17]. The control group comprised 55 female and 27 male individuals, with a mean age of 55.2 years (range, 17–85 years). All data are approved to be used for this study by the Institute of Legal Medicine, University Hospital Bonn.

The neck organs were removed in a bloodless condition. The left and right carotid bifurcations were collected at autopsy in each case. The common carotid was cut at 2 cm below the bifurcation, while the internal and external carotid arteries were cut at 2 cm above the carotid bifurcation. The excised carotid bifurcations surrounded by tissue were fixed in formalin (8%–10%). After fixation, the carotid bifurcations were laminated into thin slices (a few millimetres thick) cut in the horizontal plane (Figure 1) and then embedded in paraffin wax. Tissues were processed for haematoxylin–eosin staining, Azan and iron staining, and immunohistochemical detection of Hsp27, Hsp60, Hsp70, and AQP-3. For immunohistochemical staining, sections were first pretreated with citrate buffer (pH 6.0) for 30 min at appropriate dilutions, according to the manufacturer’s instructions (Dako LSAB2 kit or Dako Envision + kit). Sections were then incubated with mouse monoclonal antibodies against anti-Hsp27 (ab2790; Abcam, Cambridge, UK), anti-Hsp60 (H4149; Sigma-Aldrich, Saint Louis, MO, USA), or anti-Hsp70 (ab2787; Abcam) or a rabbit polyclonal antibody against AQP-3 (ab125219; Abcam) overnight at 4 °C. Sections were then incubated in appropriate secondary antibodies for 60 min at room temperature (for Hsp antibodies) or 90 min at 37 °C (for the AQP-3 antibody).

All carotid bifurcations were examined by light microscopy (Leica DM 1000, Wetzlar, Germany) for the assessment of haemorrhage and expression of Hsp27, Hsp60, Hsp70, and AQP-3 in the tissues surrounding the bifurcations, the sinus, and the carotid body (Figure 2).

Results

Among the 22 study group cases of strangulation causing death, there were 16 cases of hanging and six cases of ligature strangulation (Table 1). We hypothesised that the cases would have experienced
differing degrees of neck force (e.g. because of the rope material, rope diameter, and hanging position (upright or seated)), which would cause different pathological findings. In all cases, the deaths were caused by accidental or suicidal strangulation. No evidence of homicide was observed.

Of the 16 hanging cases, three had slight haemorrhages in the fat and connective tissues that were not in direct proximity to the carotid bifurcation (Figure 3). Fractures of the larynx and/or hyoid bone were observed in eight cases, while signs of venous stasis of the head (e.g. petechiae) were observed in 11 cases.

Of the six cases with ligature strangulation, two cases showed a slight haemorrhage—haemorrhage occurred in one case and near the carotid bifurcation in the other case (Figure 4).

Five cases showed small haemorrhages located predominantly in the surrounding fat and connective tissues. Therefore, we considered that the haemorrhages (including the haemorrhage in direct proximity to the carotid bifurcation) did not have any effects on the tissues such as severe pressure on the carotid body or sinus.

In the study group, there was no evidence of severe arteriosclerosis with vessel plaques. In four cases (Cases 3, 6, 19, and 22), the heart exceeded the critical weight of 500 g, while cases 18 and 20 showed relevant carotid bifurcation stenosis. No case showed any pathological alterations of the heart muscle. All iron stains were negative. The degree of iron-positive cells correlates with the age of the hematoma. A positive staining result that proves a vital haemorrhage requires survival for at least 3 days [18]. Thus, the negative iron staining indicated that the bleeding in all cases were rather fresh (acute bleeding) than bleeding that lasted a few days.

A range of cellular stressors can increase Hsp expression [19]. For example, Hsps can be expressed within seconds or minutes during thermal stress. Because of the temporal changes in expression of Hsps, they may be useful for determining the survival time after injury [20]. Because neck compression is also a form of external cellular stress to the neck tissues, including the carotid bifurcation, we assessed Hsp expression to examine the correlations with rapid death. However, only five cases showed positive Hsp27 staining (Figure 5), all of which were cases of hangings. Three of these cases also showed pronounced signs of congestion, indicating a longer period before cardiac failure. No cases showed positive Hsp60 or Hsp70 staining. Overall, these findings suggest that neck compression has minimal effects on Hsp expression in carotid artery tissue.

AQP-3 is considered a valuable marker for ante-mortem neck compression in the skin [21] (Figure 6). Therefore we assessed AQP-3 expression as a
Table 1. List of patients in the study group, including 22 cases with lethal ligature strangulation or hanging.

| No. | Sex   | Age (year) | Cause of death                                             | Findings of the neck                                              | Signs of venous stasis of the head | Weight of heart (g) | Stenosis bifurcation | Type of hanging |
|-----|-------|------------|------------------------------------------------------------|------------------------------------------------------------------|-----------------------------------|---------------------|---------------------|------------------|
| 1   | Female| 21         | Ligature strangulation with scarf in a seated position     | Ligature mark of the skin                                         | None                              | 250                 | –                   | –                |
| 2   | Male  | 53         | Hanging with a cable at the door and a plastic bag over the head | Ligature mark of the skin, fracture of the left superior horn of the thyroid cartilage with haemorrhage | None                              | 320                 | –                   | Complete         |
| 3   | Male  | 77         | Hanging at a tree with a tension belt                      | Ligature mark of the skin                                         | Petechiae                         | 540                 | –                   | Incomplete       |
| 4   | Male  | 10         | Hanging with a scarf at the stair railing                  | Ligature mark of the skin                                         | None                              | 190                 | –                   | Incomplete       |
| 5   | Female| 34         | Hanging with scarf and belt at the door frame              | Ligature mark of the skin, fracture of the right superior horn of the thyroid cartilage with haemorrhage | Petechiae, cyanosis                | 390                 | –                   | Incomplete       |
| 6   | Male  | 89         | Hanging                                                    | Strangulation mark of the skin, haemorrhage in the anterior musculature of the neck, fracture of the left superior horn of the thyroid cartilage with haemorrhage | None                              | 675                 | –                   | Complete         |
| 7   | Male  | 50         | Death in hospital after hanging with rope at balcony balustrade | Ligature mark of the skin, haemorrhage into both sternocleidomastoid muscles and deep layers of right musculature, haemorrhage around tissue of the left hyoid bone, fracture of the right superior horn of the thyroid cartilage with haemorrhage | Petechiae                         | 370                 | –                   | Complete         |
| 8   | Female| 58         | Hanging with nylon rope                                    | Ligature mark of the skin                                         | Petechiae                         | 330                 | –                   | Incomplete       |
| 9   | Female| 91         | Ligature strangulation with kitchen towel                  | Ligature mark of the right superior horn of the thyroid cartilage with haemorrhage | Petechiae                         | 330                 | –                   | –                |
| 10  | Female| 59         | Hanging with a belt at a cloth hook                        | Ligature mark of the skin, haemorrhage in the left sternocleidomastoid muscle | Petechiae                         | 360                 | –                   | Incomplete       |
| 11  | Male  | 54         | Hanging with a rope at a supply pipe                       | Ligature mark of the skin, haemorrhage in the anterior musculature of the neck, fracture of the left superior horn of the thyroid cartilage | Petechiae                         | 390                 | –                   | Complete         |
| 12  | Male  | 48         | Ligature strangulation with cable tie                      | Ligature mark of the skin                                         | Petechiae, cyanosis                | 400                 | –                   | –                |
| 13  | Male  | 45         | Hanging with cable at the door frame                       | Ligature mark of the skin, fracture of the left superior horn of the thyroid cartilage with haemorrhage | Petechiae                          | 300                 | –                   | Incomplete       |
| 14  | Male  | 44         | Hanging with nylon rope, fracture                          | Ligature mark of the skin, fracture of the right superior horn of the thyroid cartilage | Petechiae                          | 420                 | –                   | Incomplete       |
| 15  | Male  | 48         | Ligature strangulation with rope                            | Ligature mark of the skin, haemorrhage in the left sternocleidomastoid muscle, fracture of the right superior horn of the thyroid cartilage with haemorrhage | Petechiae, cyanosis                | 300                 | –                   | –                |
| 16  | Male  | 58         | Hanging at the window with a rope                          | Ligature mark of the skin, fracture of the right superior horn of the thyroid cartilage with haemorrhage | Petechiae                          | 250                 | –                   | Incomplete       |
| 17  | Female| 36         | Hanging with rope                                          | Ligature mark of the skin, haemorrhage subcutaneous tissue of the left posterior neck | None                              | 400                 | –                   | Complete         |
| 18  | Male  | 74         | Ligature strangulation with fixation strap at the hospital  | Haemorrhage in the left anterior musculature of the neck          | Petechiae                          | 460                 | Stenosis of the carotid bifurcation | –                |
| 19  | Male  | 51         | Hanging with rope at a ladder                              | Ligature mark of the skin, Haemorrhage at the insertion of the left sternocleidomastoid muscle at the clavicle | Petechiae, cyanosis                | 540                 | –                   | Incomplete       |
| 20  | Female| 88         | Ligature strangulation with a chain barrier                | Ligature mark of the skin, haemorrhage right stern mastoid muscle | None                              | 260                 | Stenosis (20%) of the carotid bifurcation | –                |
| 21  | Male  | 51         | Hanging with a cable                                       | Ligature mark of the skin, haemorrhage muscle of tongue right    | Petechiae                          | 390                 | –                   | Incomplete       |
| 22  | Male  | 50         | Hanging at the lattice bars of a window                    | Ligature mark of the skin                                         | Petechiae                          | 530                 | –                   | Incomplete       |
potential sign of relevant pressure on the tissue around the carotid bifurcation. However, no cases showed positive AQP-3 staining.

In the control group, no cases showed evidence of haemorrhage in carotid bifurcation tissues. Nevertheless, because of the age range (between 17 and 85 years) and variable pre-existing diseases, different stages of arteriosclerosis were found in this group.

Discussion

Whether a death-causing carotid sinus reflex can exist has been of interest to forensic scientists and lawyers for decades because of its importance for criminal law [22]. In “The van Dielingen Case” from the 1930s, a man stated that he only briefly pulled his wife by her neckcloth, whereupon she immediately collapsed and died. Because there was no evidence of ligature strangulation, the man’s sentence was reduced to 2 years for bodily harm resulting in death [23].

Many forensic pathologists agree with the theory that stimulation of the carotid bifurcation can cause death. However, to date there is insufficient evidence to support this theory. There is wide acceptance that certain conditions must occur for cardiac reflex death following violence against the neck [24]. Other causes of death and pre-existing conditions including hypotension caused by cardiac arrhythmia, digitalis medication, or atherosclerosis should be excluded [10]. Furthermore, a history of rapid death or immediate loss of consciousness should be considered, including signs of rapid cardiovascular system failure.

Two cases in our study group (74 and 88 years old) showed evidence of relevant carotid bifurcation stenosis. Sigrist et al. [25] were the first to report a diagnosis of sudden death after direct impact to the neck, with seven cases of rapid death due to reflex zone injury (Table 2). In their study, diagnosis required evidence of injury to the reflex zone at autopsy, as well as ruling out of signs of acute cardiovascular failure and other causes of death. By contrast, there was no evidence of rapid death in the history of our cases. In 20 of the 22 cases, autopsy revealed signs of venous stasis or muscular haemorrhage, indicating longer preservation of cardiocirculatory activity and vital reaction signs, which excludes immediate reflex death.

The present study aimed to assess morphologically detectable injuries, particularly haemorrhages, in the region of the carotid bifurcation after ligature strangulation and hanging, which may have a relevant effect on the reflex zone. This type of haemorrhage was previously described in “The Deadly Broomstick Case”, in which a 51-year-old man was hit by a broomstick on the left side of his neck, after which he collapsed and died. Autopsy revealed a severe haemorrhagic compression of the carotid bifurcation. The authors suggested that this was a reflexogenic incident because of his immediate collapse. Because the haemorrhage surrounded the carotid bifurcation, and thus the carotid sinus, ongoing compression with a reflex causing prolonged cardiodepression were assumed. Without ongoing compression of the neck reflexogenic zones caused by the severe haemorrhage, his collapse following bradycardia and vasodilation may have been self-limiting [26].
Table 2. Seven cases of rapid death after intensive impact and injury to the reflex zone of the neck. Adapted with permission: [15].

| Sex/age (year), occupation | Accident | Findings in the neck | Other findings | BAC (%) |
|---------------------------|----------|----------------------|----------------|---------|
| Male/50, farmer           | Hit by a hoof of a cow against the left side of the neck | Contusion of the skin, haemorrhage of the left neck, laceration of the intima of the carotid sinus, haemorrhage of the adventitia, and fracture of the upper horn of the left larynx | Acute cardiac dilatation, hyperaemia of all organs, and slight coronary arteriosclerosis | Negative |
| Female/63, landlady       | Fall to the ground, hit in the neck by the back of a chair | Haemorrhage in subcutaneous tissue and musculature in the front and side of the neck, intramural haemorrhage at the left carotid bifurcation, fracture of the left hyoid bone, and fracture of the right upper horn of the larynx | Acute cardiac dilatation, hyperaemia of inner organs, slight coronary arteriosclerosis, and arteriosclerotic shrinking of the right kidney | Negative |
| Female/26, housewife      | Fall from a motorbike, hit by the rim of a helmet against the neck | Abrasion at the chin and neck, haemorrhage of the neck soft tissue, and lacerations of both carotid arteries in the height of the bifurcation | Contusion of the lumbar soft tissue, rib fractures, lacerations of the lungs by rib fractures, and fractures of the arms | Negative |
| Male/25, carpenter        | Entrapment between the ground and edge of the roof of an overturned car | Abrasions of the neck, haemorrhage of the neck muscles, subadventitial haemorrhage of the left carotid artery, and subperioisteal haemorrhage at the larynx | Further abrasions at the head and hyperaemia of inner organs | 0.7 |
| Female/20, unknown        | Karate hit with the hand against the neck. Removal of the body | Fracture of the right upper horn of the larynx and slight haemorrhage | Criminal dismemberment of the body (no vital reactions) and putrefaction | 0.3 |
| Male/74, farmer           | Entrapment of the neck by a trailer of a tractor | Abrasion of the neck, subcutaneous contusions, slight haemorrhage of the neck muscle on the left side, and haemorrhage of the carotid sinus on both sides | Slight coronary arteriosclerosis and left ventricular hypotrophy | 0.54 |
| Male/57, unemployed        | Dispute in a home for men. Hit with a crutch against the neck and thorax | Haemorrhage of the neck in the front and on the left side, fracture of the larynx on the left side, laceration of the intima, and haemorrhage of the wall of the left carotid sinus | Lacerations on the head, chest, and extremities, advanced coronary arteriosclerosis, scar of myocardial infarction, bronchitis, pulmonary emphysema, and alcoholic liver damage | 2.98 |

FE: pulmonary fat embolism; BAC: blood alcohol concentration.
the neck. A reflex death was assumed to have occurred in these cases because of the rapid onset of death, rather than the presence of haemorrhages.

In the present study, no cases showed any positive AQP-3 staining. AQP-3 is a major water channel associated with the skin and plays a major role in water reabsorption, including replacement of water loss due to evaporation. AQP-3 is expressed in the skin of ligature marks and is considered a reliable marker of antemortem neck compression [21]. AQP-3 is also expressed in other cell types including adipocytes, astrocytes, and endothelial cells [21]. Because AQP-3 can be induced in the skin following compression, we examined AQP-3 expression in the carotid bifurcation and surrounding tissue. However, our findings suggest that AQP-3 staining is not a useful marker for relevant neck pressure, or that there was actually no relevant neck impact. Further studies are required to confirm these findings, including more cases with different types of neck impacts and with signs of rapid death.

Finally, our findings provide no direct evidence for reflex cardiac death resulting from a brief force against the neck. Nevertheless, in selected cases with a suitable history, the potential for reflex death following intense force against the neck should still be considered. However, other causes of death should be ruled out by histological, morphological, and toxicological investigations, while morphological findings, including relevant haemorrhages in the carotid artery tissues and signs of prolonged survival and vital reactions, should be detected [25]. Notably an absence of haemorrhages does not rule out reflex death. Therefore, other markers are required to indicate a relevant pressure to the carotid bifurcation region. Without these conditions, a diagnosis of reflex death is not appropriate.

Authors’ contributions
Julia Ulbricht carried out the histological analyses and drafted the manuscript; Elke Doberentz and Burkhard Madea conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors contributed to the final text and approved it.

Compliance with ethical standards
This article does not contain any studies with human participants or animals. This study does not need IRB approval according to the the statements by the Central Ethics Committee of the of the German Medical Association (ZEKO) on (1) the (further) use of human body materials for the purposes of medical research (https://www.zentrale-etikkommission.de/stellungnahmen/koerpermaterialien, (24. 6. 2021), DÄBl. 2003, A1632), and (2) the principles of which have been supplemented to include the use of body materials of deceased persons (Die (Weiter-)Verwendung von menschlichen Körpermaterialien von Verstorbenen für Zwecke medizinischer Forschung, DÄBl. 2003, A2251).

Disclosure statement
The authors report there are no competing interests to declare.

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