Forensic Science, Medicine and Pathology
https://doi.org/10.1007/s12024-024-00934-0

REVIEW

# Immunohistochemical insights into hypothermia-related deaths: a systematic review

**Luca Tomassini[1] · Massimo Lancia[2] · Cristiana Gambelunghe[2] · Giulia Ricchezze[3] · Piergiorgio Fedeli[4] · Mariano Cingolani[3] · Francesco De Micco[5] · Elena Fiorucci[2] · Roberto Scendoni[3]**

Accepted: 17 December 2024
© The Author(s) 2024

## Abstract

**Purpose** Hypothermia occurs when core body temperature drops below 35 °C. The purpose of this review was to identify and analyze studies on the topic of hypothermia from an immunohistochemical perspective to determine robust markers of fatal hypothermia.

**Methods** This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines. It has been registered with Prospero (registration number: CRD42024572782).

**Results** In total, 2618 publications met the search criteria. A total of 63 duplicate articles were excluded and a further 2489 publications were excluded for not meeting the inclusion criteria, leaving 29 full-text articles. Furthermore, from the bibliographies of the included articles, four more publications were selected, which also respected the established inclusion and exclusion criteria. Only six studies identified a statistically significant correlation between immunohistochemical patterns and hypothermia-related deaths.

**Conclusion** The existing literature examined appears fragmented and lacks robust statistical validation. Therefore, there is an evident need for more comprehensive and methodologically rigorous research to develop reliable diagnostic criteria.

**Keywords** Hypothermia · Immunohistochemistry · Forensic autopsy · Cold-related deaths · Parenchymal analysis.

✉ Giulia Ricchezze
g.ricchezze1@unimc.it

Luca Tomassini
luca.tomassini@unicam.it

Massimo Lancia
massimo.lancia@unipg.it

Cristiana Gambelunghe
cristiana.gambelunghe@unipg.it

Piergiorgio Fedeli
piergiorgio.fedeli@unicam.it

Mariano Cingolani
mariano.cingolani@unimc.it

Francesco De Micco
f.demicco@unicampus.it

Elena Fiorucci
fioruccielena98@gmail.com

Roberto Scendoni
r.scendoni@unimc.it

[1] International School of Advanced Studies, University of Camerino, Camerino, Italy

[2] Forensic Medicine, Forensic Science and Sports Medicine Section, Department of Medicine and Surgery, University of Perugia, Perugia, Italy

[3] Department of Law, Institute of Legal Medicine, University of Macerata, Macerata, Italy

[4] School of Law, Legal Medicine, University of Camerino, Camerino, Italy

[5] Research Unit of Bioethics and Humanities, Department of Medicine and Surgery, Università Campus Bio-Medico di Roma, Rome, Italy

Published online: 09 January 2025

## Introduction

Hypothermia is defined as a decrease in core body temperature below 35 °C, resulting from an imbalance where heat loss exceeds heat production [1]. It is a significant cause of mortality in extreme environments, such as mountainous or marine settings [2]. In these cases, it is referred to as exogenous hypothermia, as distinct from endogenous hypothermia and from that associated with hypothyroidism [3].

In addition, hypothermia can develop in elderly or debilitated individuals within controlled environments, such as their homes, through a combination of exogenous and endogenous factors [3]. This is known as accidental hypothermia. Typically, in response to cold exposure the body undergoes a series of endocrine and metabolic changes depending on the drop in body temperature. Risk of death has been described at over 70% for core body temperatures of 30 °C and over 90% for core body temperatures of 26 °C [4, 5].

Macroscopic observations in cases of fatal hypothermia include Wischnewsky spots, erythema, and hemorrhaging in the iliopsoas muscles [6–8]. Among the most commonly documented histological findings in hypothermia cases are fatty degeneration of myocytes, epithelial cells in the proximal renal tubules, and pancreatic cells [1, 3, 6].

While certain characteristic signs of hypothermic death are recognized, they are not consistently present in all cases. Furthermore, the absence of definitive findings at autopsy often underscores the critical importance of the deceased's medical and circumstantial history in establishing a diagnosis [9]. This situation raises the critical question of how much a pathologist can rely on circumstantial evidence instead of objective findings of their examination, when determining the cause of death [10, 11].

Consequently, hypothermia represents one of the most intricate dilemmas in pathology. This is comparable to certain cases of epilepsy, asthma, and drowning. The challenge is especially significant when autopsy findings are minimal, non-specific, or entirely absent [12]. For this reason, immunohistochemistry has been considered a potentially useful tool in hypothermia diagnosis [13–15].

The aim of this systematic review was to identify and analyze studies in which hypothermia has been investigated, either partially or exclusively, through immunohistochemical analysis. It sought to determine whether, based on the available literature, there is robust evidence to support the use of immunohistochemistry for diagnosing hypothermia using the various markers examined. The overall objective was to assess whether immunohistochemistry can be considered a practically applicable tool in the diagnosis of hypothermia-related death.

## Materials and methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines. It has been registered with Prospero (registration number: CRD42024572782).

The PRISMA checklist is provided as supplementary material.

A systematic literature search was conducted by PubMed, Scopus, and Web of Science to retrieve studies published in English on immunohistochemical techniques used to determine a postmortem diagnosis of hypothermia.

The generic free-text search terms were: ("Hypothermia" [All Fields] OR "accidental hypothermia" [All Fields] OR "fatal hypothermia" [All Fields] OR "cold-related deaths" [All Fields]) AND ("immunohistochemical" [All Fields]). Filters applied were: "Humans" and "English". No time limit was given to this research.

Two researchers independently searched PubMed, Scopus, and Web of Science for studies, while three other researchers checked whether the selected articles met the inclusion criteria.

The following data were recorded from the chosen studies: authors and year of publication, country of affiliation, type of article, number of case-controls, type of study, type of population investigated, type of parenchyma/organ studied, marker studied, histochemical staining used, type of statistical analysis performed, and statistical results obtained. The resulting documents underwent another round of screening based on the language (excluding non-English texts), title, abstract, methods, and keywords.

The selection process was carried out using Rayyan, a free web and mobile application that expedites the initial screening of abstracts and titles through a semi-automated process. It incorporates a high level of usability and can be accessed at the following website: http://rayyan.qcri.org [16, 17].

The publications finally selected for analysis had to respect the following inclusion criteria:

- post-mortem investigations;
- forensic study;
- study of human tissues;
- study about immunohistochemistry.

Non-inclusion and exclusion criteria were:

- hypothermia treated in a clinical setting;
- complications due to exposure to low temperatures;
- clinical management;
- critical patient;
- systematic review;

- studies related to techniques not involving immunohistochemistry.

Other relevant articles that met the inclusion and exclusion criteria were extrapolated from the bibliography of articles generated by the generic free-text search.

The study was designed according to PRISMA recommendations and descriptive statistics were used to organize the data. The selected articles were analyzed for the scope of immunohistochemistry used in cases of hypothermia, the tissues used, and the markers sought. In addition, information was collected on countries and dates of publication.

The data collection process included both the selection of studies and data extraction.

As mentioned previously, three researchers independently assessed whether the articles had titles or abstracts that met the inclusion criteria, and any disagreements were resolved by achieving consensus. Two researchers extracted the relevant data, which was then reviewed by two other researchers and subsequently reconfirmed by an additional pair of investigators.

It should be noted that in the case of studies involving both immunohistochemistry and other investigative techniques (e.g., biomarker concentrations in body fluids), the latter are noted in the results section.

## Results

In total, 2618 publications met the search criteria.

A total of 63 duplicate articles were excluded. After consideration of whether studies met the primary inclusion criteria, a further 2489 publications were excluded, leaving 29 full-text articles. Upon reviewing the full-text articles, an additional 13 studies were excluded for not adhering to the inclusion criteria. The remaining 16 full-text articles therefore fully met the inclusion criteria for the review. Furthermore, from the bibliographies of the included articles, four more publications were selected, which also respected the established inclusion and exclusion criteria. The article selection process is summarized in Fig. 1.

Out of the total 20 studies, 13 investigated hypothermia cases, while seven also took into consideration hyperthermia cases.

Focusing on immunohistochemistry, 11 studies exclusively examined these techniques, while nine studies incorporated both immunohistochemistry and other investigative

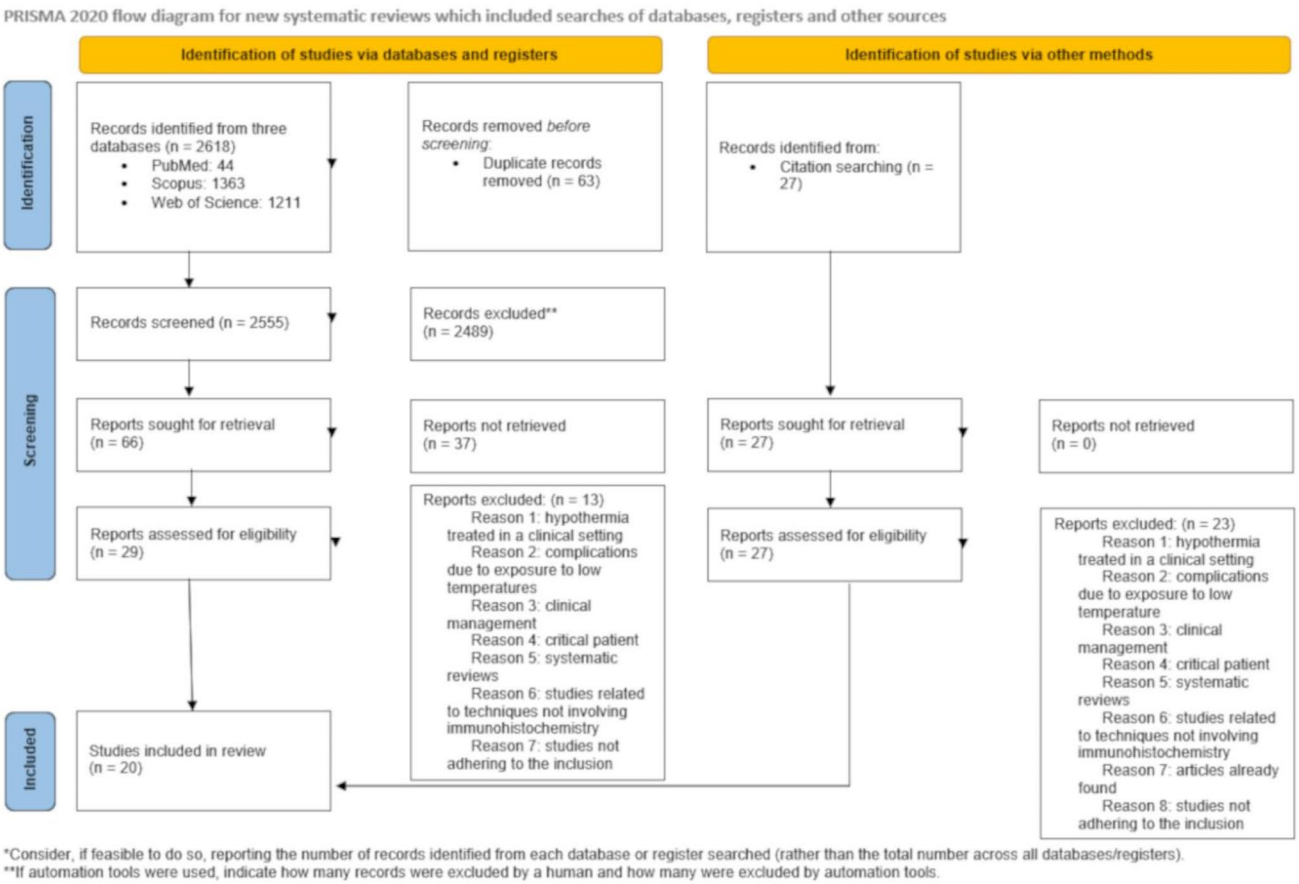


**Fig. 1** Descriptive diagram of the paper selection process

techniques, such as molecular analysis, gene expression studies, and biochemical assays.

The studies analyzed various types of parenchyma and organs: the pituitary gland was examined in eight studies, the kidney in five studies, and the myocardium in four studies. The lungs were analyzed in three studies. The hippocampus, hypothalamus, adrenal glands, encephalon, pancreas, and liver were included in two studies each. Finally, the spleen, gastric mucosa, and cerebellum were each covered in one study.

A wide range of immunohistochemical markers was investigated across the 20 studies, notably the following: Adrenocorticotropic hormone (ACTH) appeared in four studies, heat shock protein 70 (Hsp70) in three studies, and catecholamines (including adrenaline, noradrenaline, and dopamine) in one study. Additionally, phosphorylated heat shock factor 1 (HSF1), chromogranin A (CgA), and thyroid-stimulating hormone (TSH) each featured in two studies.

Other markers that were evaluated, each in a single study, included aquaporin 1 (AQP-1), aquaporin-5 (AQP-5), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), messenger ribonucleic acid (mRNA), C5b9, claudin-5 (CLDN-5), glial fibrillary acidic protein (GFAP), hemoglobin (Hb), heat shock protein 27 (Hsp27), intercellular adhesion molecule 1 (ICAM-1), interleukin-1β (IL-1β), interleukin-10 (IL-10), matrix metallopeptidase 2 (MMP-2), matrix metallopeptidase 9 (MMP-9), microtubule-associated protein 2 (MAP2), S100b, ssDNA, lung surfactant A (SP-A), lung surfactant D (SP-D), sirtuin 1 (SIRT1), tumor necrosis factor α (TNF-α), thrombomodulin (TM), ubiquitin (Ub), growth hormone (GH), gonadotropins, prolactin, and basic fibroblast growth factor (bFGF).

The statistical analyses commonly employed included: the Mann-Whitney U test (used in seven studies), Fisher's Exact Test (five studies), the Scheffe test (four studies), ROC curves (four studies), the Bonferroni correction (three studies), the Kruskal-Wallis test (three studies), ANOVA (Analysis of Variance) (two studies), Spearman's correlation coefficient (two studies), the Tukey test (two studies), the Steel-Dwass test (two studies), and the chi-square test (one study). Others used were: the Benjamini-Hochberg procedure, Student's t-test, Levene's test, the one-tailed test, the non-parametric test, Pearson's correlation coefficient, descriptive analysis, and regression analysis (each of these was used in a single work).

Six studies (30%) found a statistically significant correlation between immunohistochemical expressions and hypothermia-related deaths. Conversely, in 14 studies (70%), no statistical significance was observed between the expression of a specific immunohistochemical marker and death from hypothermia, or no proper statistical analysis was conducted within the study.

The studies that demonstrated a statistically significant correlation of immunohistochemical markers concerned: anti-thrombomodulin in the myocardium [18]; anti-TSH in the pituitary gland [19]; the expression of chromogranin (CgA) in hypothalamic neurons, the adenohypophysis, and the adrenal glands [14]; the expression of anti-Hsp 70 positivity in renal podocytes [20]; the positivity of anti-bFGF and S100b in glial cells [21]; and anti-ACTH in the anterior pituitary gland [22].

A summary of the results of the individual studies are presented in Table 1. A more detailed table containing all the data extrapolated from each study can be found in Supplementary Material. In addition, the results are further schematized in graphical form in Fig. 2.

## Discussion

Determining hypothermia as a cause of death is challenging due to non-specific, inconsistent, or negative autopsy findings [23]. The most diagnostically significant macroscopic alterations were first documented by Keferstein (frost erythema), Wischnewsky (hemorrhagic lesions of the gastric mucosa), and Krjukoff in the late nineteenth and early twentieth centuries [23, 24]. Despite extensive research efforts over the years aimed at establishing more reliable diagnostic criteria, progress in the accurate identification of hypothermia as a cause of death has remained relatively limited [1, 23].

In this context, the potential application of relatively novel immunohistochemical techniques in diagnosing hypothermia-related deaths warrants consideration, given the distinct adaptive changes and stress responses induced by hypothermic conditions. These techniques offer the possibility of detecting specific molecular and cellular alterations that may provide critical insights into the physiological impact of hypothermia, even in the absence of overt macroscopic or histological findings [6, 21, 23].

The data analyzed various organs and tissues across multiple studies, indicating a broad approach to identifying potential immunohistochemical markers in cases of death from hypothermia [25].

Key organs such as the pituitary gland, kidneys, myocardium, and lungs were more frequently studied, while other organs like the hippocampus, hypothalamus, and adrenal glands received comparatively less attention. Thus, the primary focus has been on organs potentially more susceptible to hypothermic stress responses.

Certain markers tend to be associated with both hyperthermia and hypothermia diagnostics, and more generally with stress responses, but this renders these markers non-specific [15, 26–28]. In fact, it is pointed out that many of

**Table 1** Synthesis table of the studies analyzed

| Authors and year of publication | Number of case/ control (if applicable) | Type of parenchyma/ organ studied | Marker studied | Results |
|---|---|---|---|---|
| Lasse Pakanen et al. (2014) [18] | Cases: 106 Controls: 164 | Myocardium (anterior wall of left ventricle) | Thrombomodulin (TM) | The study also examined TM protein levels in myocardial tissue, finding that hypothermia groups had significantly lower levels of TM staining in intra-myocardial capillaries and arterioles/venules compared to controls. |
| Chen JH et al. (2012) [36] | Cases: Controls: 46 | Myocardium | Atrial and brain natriuretic peptides (ANP and BNP) | Immunostaining showed ANP and BNP in cardiomyocytes, with staining intensity varying among cases, but with no obvious differences between causes of death. |
| Takaki Ishikawa et al. (2009) [19] | Cases: 120 | Pituitary gland | Thyroid-stimulating hormone (TSH) | TSH immunopositivity in the pituitary is significantly lower in hypothermia cases than in other causes of death. There is no correlation between TSH immunopositivity in the pituitary and TSH levels in serum or CSF. |
| T. Ishikawa et al. (2004) [27] | Cases: 31 Controls: 180 | Anterior lobe of the pituitary gland (adenohypophysis) | ACTH (Adrenocorticotropic hormone) Gonadotropins TSH (Thyroid stimulating hormone) GH (Growth hormone) Prolactin | Cytoplasmic vacuoles found in about 40% of adenohypophysis cells in fatal hypothermia cases. Detection rate less than 1% in the other cases of death. Frequency of vacuolation highest in ACTH cells (about 65%), followed by gonadotropic (about 43%) and lowest in TSH cells (about 16%). |
| Hleşcu AA et al. (2022) [32] | Cases: 107 hypothermia-related deaths | Myocardium and kidney | Sirtuin 1 (SIRT1) in myocardium, Ubiquitin (Ub) in kidneys | Myocardium: SIRT1 expression was weak, with a loss of immunopositivity in areas with contraction bands, suggesting association with hypothermia-induced myocardial changes. |
| Doberentz E et al. (2017) [6] | Cases: 11 Controls: 10 | Pituitary gland | Hsp27, Hsp70 | Expression of Hsp27 was detected in only a minority of the incidental hypothermia cases, with weak staining. No expression of Hsp70 was found in the hypothermia cases. Fatty degeneration of pituitary gland cells was more common in hypothermia cases than in controls but showed no significant correlation with the expression of Hsp27 and Hsp70. |
| Miyazato T et al. (2012) [37] | Cases: 56 Controls: 63 | Lung tissue | SP-A (Lung Surfactant A), SP-D (Lung Surfactant D), TNF-α (Tumor necrosis factor α), IL-1β (Interleukin-1β), IL-10 (Interleukin-10). | Variations in immunopositivity for SP-A, SP-D, IL-1β and TNF-α among different causes of death were significant. Although the specific description of the statistical tests was not provided in the text, it is reasonable to assume that statistical analysis was conducted to confirm the significance of the results. |
| Takaki Ishikawa et al. (2010) [38] | Cases: 32 Controls: 258 | Hypothalamus, pituitary and adrenal glands | Adrenaline, noradrenaline and dopamine | In the hypothalamus, immunocytochemical positivity of catecholamines was found mainly in neuronal cells, without significant interstitial staining. Other regions show both interstitial and neuronal staining. No links were found between immunocytochemical positivity and postmortem period, age, or gender of the subjects. In the hypothalamus, neuronal positivity for dopamine is significantly lower for hyperthermia than for hypothermia and other groups. In adenohypophysis, positivity for dopamine is lower in cases of sudden cardiac death than in other causes, except for hyperthermia. In the adrenal medulla, positivity for noradrenaline and dopamine is higher in cases of hyperthermia than in other causes, including hypothermia. Positivity for adrenaline is lower in cases of fire death than in other causes, but there are no significant differences between hypothermia and hyperthermia. |

**Table 1** (continued)

| Authors and year of publication | Number of case/ control (if applicable) | Type of parenchyma/ organ studied | Marker studied | Results |
|---|---|---|---|---|
| Chiemi Yoshida et al. (2009) [15] | Cases: 20 Controls: 298 | Hypothalamus, adenohypophysis (anterior pituitary gland), adrenal medulla | Chromogranin A (CgA). Total numbers of neurons in the hypothalamus and endocrine cells in the adenohypophysis and adrenal medulla and the number of cells showing CgA immunoreactivity were counted under ×200 magnification: Five random fields were independently examined by two observers using the Lumina Vision system according to standard procedures, and mean values were estimated. | CgA immunopositivity in the adenohypophysis and adrenal medulla was similar for each cause of death. Hypothalamus neuronal CgA immunopositivity showed an extensive variation among cases in each cause of death, but was lower in most cases of hypothermia, compared with other groups including intoxication and hyperthermia. CgA immunopositivity showed a positive correlation with the CSF CgA level for hypothermia. However, CgA immunopositivity in the adenohypophysis was higher for asphyxia than in any other group, excluding intoxication and hypothermia. CgA immunopositivity in the adrenal medulla was independent of the cause of death. No significant relationship was detected between CgA immunopositivity in the adenohypophysis or adrenal medulla and serum or CSF CgA levels. |
| Qi Wang et al. (2013) [39] | Cases: 122 | Upper lobe of the left lung | MMP-2 (Matrix Metallopeptidase 2), MMP-9 (Matrix Metallopeptidase 9), ICAM-1 (Intercellular Adhesion Molecule 1), CLDN-5 (Claudin-5), AQP-1 (Aquaporin-1), AQP-5 (Aquaporin-5) | MMP-2 was detected mainly in epithelial cells, with higher intensity in hyperthermia cases. MMP-9 was detected in interstitial cells, mainly in macrophages, with more intense staining in hyperthermia and hypothermia. ICAM-1 was diffusely detected in alveolar walls, with no significant differences in distribution or intensity among causes of death. CLDN-5 was strongly positive in bronchial epithelia and capillary endothelial cells, with no significant differences in distribution or intensity among causes of death. AQP-1 was clearly positive in all vascular endothelial cells, with no significant differences in distribution or intensity among causes of death. AQP-5 was positive in a linear pattern in type I alveolar epithelial cells and bronchial epithelial cells, with granular aggregates and fragments more frequent in the intra-alveolar spaces in cases of atypical hanging. |
| Alice Natanti et al. (2021) [13] | Cases: 23 (11 fatal hypothermia, 4 fatal hyperthermia) Controls: 8 | Liver, pancreas, kidney | Chromogranin A (CgA) in the pancreas | CgA was markedly positive in the pancreatic tissue of five cases of fatal hypothermia. All samples collected in drowning-related deaths showed the intense positivity of the islets of Langerhans to CgA, and the islets appeared clearly distinguishable. |

**Table 1** (continued)

| Authors and year of publication | Number of case/ control (if applicable) | Type of parenchyma/ organ studied | Marker studied | Results |
|---|---|---|---|---|
| Michael Tsokos et al. (2006) [40] | Cases: 14 fatal hypothermia Controls: 10 | Gastric mucosa | Hemoglobin (Hb) | Lesions identified as Wischnewsky spots in kerosene blocks react positively to anti hemoglobin antibodies. This indicates a hemorrhagic nature of the lesions, with a diffuse staining pattern that does not correspond to intact erythrocytes. In controls with erosive gastritis, extravasated erythrocytes react positively with anti-hemoglobin antibodies. No positivity for hemoglobin in controls with normal gastric mucosa. |
| Sakurada et al. (2012) [20] | Cases: 17 Controls: 29 | Kidneys | HSP70, Phosphorylated Heat Shock Factor 1 (HSF1) | HSP70 was present in almost all samples, though the intensity of expression varied. The immunohistochemical analysis identified podocytes as the primary glomerular cell type expressing HSP70. In some cases, epithelial cells of Bowman's capsule also showed HSP70 expression, but no other cell types exhibited significant labeling. The cold-temperature group exhibited a unique expression pattern in 14 out of 15 samples, distinguishing it from the hot and normal temperature groups, which were harder to differentiate based on HSP70 expression. No significant correlations were found between the degree of HSP70 expression and variables such as age, sex, or postmortem interval. However, in samples categorized under pattern II, the intensity of HSP70 expression varied significantly, with some cases involving drug abuse or fire victims (from the normal and hot temperature groups) showing very strong expression in both the cytoplasm and nucleus. |
| Doberentz et al. (2011) [41] | Cases: 17 hypothermia Controls: 61 | Pituitary glands | C5b9, adrenocorticotropic hormone (ACTH) | The immunohistochemical examination with LCA-staining showed few leukocytes in the anterior lobe structures: in two cases of the study group and 25 cases of the control group. The red colored positively identified leukocytes within the tissue can be seen. Positively identified C5b9 complement membrane attack complexes as necrosis marker was not found within the anterior pituitary of both groups. ACTH-immunostaining was positive in all anterior pituitary cells of the study group and the control group. In almost half of the investigated cases of fatal hypothermia, hyperemic tissue of the adenohypophysis was found. |
| Preuß et al. (2007) [42] | Cases: 100 Controls: 50 | Kidneys | HSP70 | 89.0% of the cases in the study group presented signs of HSP 70 expression in the tubule epithelium cells of different grades. The HSP70 expression in the tubular epithelium cells could be detected both in the cytoplasm and nucleus of the cells, but mostly as a granular pattern in the cytoplasm. |
| Wang et al. (2012) [21] | Cases: 33 Controls: 39 | Parietal cortical layers III–V and white matter and hippocampal CA4 region of left cerebral hemispheres | bFGF, GFAP, S100b and ssDNA | There was no difference in the numbers of glia cells and neurons among all groups. bFGF levels in glial cells of the parietal cerebral cortex were significantly higher in cases of hyperthermia compared to IHD and asphyxial death, and higher in both the parietal cortex and white matter in hypothermia compared to hyperthermia and control cases. However, no significant difference was detected in the hippocampus. Regarding GFAP, there was no difference in glial GFAP immunopositivity across the causes of death in the parietal cortex and hippocampus. However, in the cerebral white matter, GFAP immunopositivity was lower in hyperthermia compared to hypothermia and control cases. S100b levels were higher in the parietal cortex during hyperthermia compared to IHD and drowning, and higher in hypothermia compared to all control groups. In the parietal white matter, S100b levels were lower during hyperthermia compared to hypothermia and control cases. Nevertheless, ssDNA immunopositivity in the parietal cortex and hippocampus was higher in hyperthermia compared to hypothermia and control cases. |
| Shimizu et al. (1997) [43] | Cases: 11 Controls: 5 | Liver, kidney, lung, heart, pancreas, spleen, brain and cerebellum | Ubiquitin (Ub) | Ubiquitin expression was mainly detected in epithelial cells of various organs, suggesting significant cellular stress caused by low temperatures. In the liver, ubiquitin expression was found in many bile duct epithelial cells and in some cases in the hepatocellular nuclei, while in the kidneys in the cytoplasm and nuclei of tubular epithelial cells. In the lungs, expression has been shown in the nuclei of alveolar epithelial cells and in some samples in bronchial epithelial cells, while in the pancreas it is expressed in part of the exocrine cells and in many endocrine cells of the islets of Langerhans. In the heart, expression of ubiquitin was found in the nuclei of muscle fibers in half of the samples, while in the spleen and brain it was not found at expression. |

**Table 1** (continued)

| Authors and year of publication | Number of case/control (if applicable) | Type of parenchyma/organ studied | Marker studied | Results |
|---|---|---|---|---|
| Ishikawa et al. (2008) [22] | Cases: 18<br>Controls: 144 | Anterior pituitary | ACTH | Firstly, the analysis revealed no significant differences in serum and CSF ACTH levels based on gender or age. A strong negative correlation was observed for hypothermia, indicating that CSF ACTH levels tend to decrease as the survival period increases in hypothermia cases. Regarding the cause of death, the study found that serum ACTH levels in cases of sharp instrument injury, fire fatalities, drowning, and hypothermia were within or above the clinical reference range. |
| Doberentz et al. (2017) [8] | Cases: 34<br>Controls: 61 | Anterior pituitary gland, gastric mucosa | Hyperemia, hemorrhage, cell vacuolization, leukocytes (LCA), ACTH, membrane complex C5b-9 | Leukocytes (LCA) stained positively in 2 cases in the fatal hypothermia group and in 25 cases in the control group. ACTH gave positivity in all cases in both groups with similar expression patterns. |
| Kitamura et al. (2005) [44] | Cases: 8<br>Controls: 12 | Hippocampus | Microtubule-associated protein 2 (MAP2) | The hypothermic group showed better preservation of MAP2 immunoreactivity in the dendrites of the CA1-subiculum neurons. However, there was more evident cytoplasmic accumulation of MAP2, particularly near the stratum oriens. In other regions of the hippocampus, such as CA2–CA4 and the dentate gyrus, the differences between the two groups were less significant. In these areas, MAP2 staining was maintained in both cell bodies and dendrites, and the changes were less severe compared to those observed in the CA1-subiculum. The dentate gyrus retained intense MAP2 staining in the molecular layer and granule cells across both groups. |

the markers analyzed by the different studies and shown in Fig. 2 are stress-response factors, such as HSP, S100b, ubiquitin ACTH, and, as a result, they are inevitably exacerbated by aspecificity. Therefore, it would be worthwhile to investigate future research toward more specific markers for hypothermia, such as response hormones produced by endocrine organs [29–31].

A diverse array of immunohistochemical markers was investigated, with some, such as ACTH, Hsp70, and catecholamines, receiving attention in different studies [6, 27]. However, many markers were studied in isolation, reflecting a fragmented research approach where individual studies have focused on specific biological pathways or markers, rather than a comprehensive examination of a broader range of markers across multiple studies.

It should be noted that in the majority of cases, immunohistochemistry was performed in combination with other techniques (such as mRNA expression and the measurement of markers in biological fluids). Therefore, many studies did not initially regard immunohistochemistry as a standalone diagnostic tool; this aspect must be acknowledged as a limitation of the present work.

To reiterate: out of the 20 studies resulting from the selection process, only six demonstrated some degree of statistical significance concerning the usefulness of immunohistochemical techniques in the diagnosis of fatal hypothermia, excluding other investigations conducted in the study.

The diagnosis of death by hypothermia, like other death diagnoses, can hold significant value in legal proceedings. Therefore, highly reliable, scientifically validated diagnostic tools are needed to present strong evidence before a judge [28].

Additionally, it should be emphasized that each of the six studies showing some type of statistically significant correlation involved a different immunohistochemical marker, often in different tissues, with correlations that were sometimes only weakly significant, as observed in the studies by Ishikawa et al. (2008) and Pakanen et al. (2015) [18, 22]. This raises doubts about the practical applicability of such findings in the diagnosis of death by hypothermia within forensic practice.

Furthermore, Hleșcu's study considered a population consisting solely of hypothermia cases, without comparison with a control group of subjects who died from other causes [32].

It can thus be inferred from the results of the present work that the existing evidence is inconsistent and lacks solid statistical support, even though recent developments in immunohistochemical methods present opportunities for enhancing the accuracy of hypothermia diagnosis in death investigations. The variability and limited reproducibility

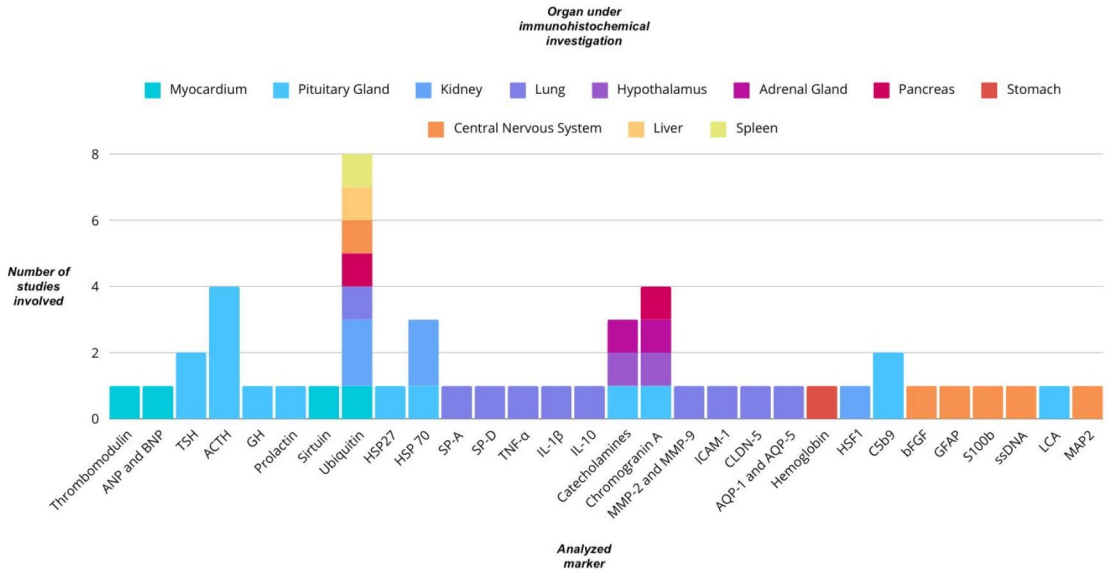


**Fig. 2** Bar graph summary of the results obtained

seen in previous studies indicate a pressing need for more thorough and methodologically sound research to establish dependable diagnostic standards [33].

Until such detailed studies are carried out, the practical use of these immunohistochemical markers in forensic practice remains questionable [34]. Therefore, it is crucial to persist in advancing and refining diagnostic strategies to ensure that forensic assessments are based on scientifically validated and dependable evidence [35].

## Limitations

The main limitation of this review is that there are very few papers, heterogeneous with each other, dealing with the applicability of immunohistochemistry to hypothermia-related deaths. This lack makes it difficult to use this technique practically applicable.

Another primary limitation lies in the fact that the statistical analysis performed in the various studies predominantly relied on descriptive statistics, without incorporating more advanced statistical models such as multivariate analyses, regression tests, or predictive models. The approach adopted in much of the literature, partly dictated by the nature of the methodology employed, effectively restricts the ability to identify causal or significant relationships among the variables studied and significantly limits the potential to generalize the findings to a broader context.

## Perspectives

The findings from this systematic review highlight the fragmented nature of current knowledge and underscore the need for a more systematic and integrated approach in research on the postmortem diagnosis of hypothermia. The results obtained indicate that in the event of a continuation of immunohistochemistry in the study of hypothermia death, scientific studies should take the following steps:

- An important prospect for future research is to expand the investigation to a broad range of immunohistochemical markers by adopting an integrative approach involving multiple organs and parenchyma. Combined analysis of specific and nonspecific markers could represent a significant methodological advance, improving diagnostic sensitivity and specificity. Such a strategy would overcome the limitations associated with the isolated examination of single markers, providing a completer and more reliable picture for the postmortem diagnosis of hypothermia.
- It is imperative that future research be based on rigorous and carefully designed statistical methodologies capable of isolating and controlling potential confounding factors that might influence the results. Such an approach is essential to ensure the reliability and reproducibility of analyses, allowing biological and molecular changes specifically associated with hypothermia to be clearly

and unequivocally distinguished from those attributable to alternative dying conditions. The adoption of advanced statistical tools, combined with meticulous experimental planning, will make it possible to assess the complex interactions between biological markers, demographic variables, and environmental circumstances. In fact, recent studies seem to be moving toward a greater trend toward the use of statistical tools.

- The presence of appropriate control groups, representative of other dying conditions, is crucial to verify the specificity of the identified markers. This aspect is often overlooked, leading to conclusions that are potentially limited or not applicable to forensic practice.
- Future research, such as the proposal of specific markers for further investigation or the development and implementation of standardized protocols for immunohistochemical analysis in cases of suspected hypothermia, would be highly beneficial in advancing the field. These efforts could help establish more reliable diagnostic criteria and enhance the overall accuracy and reproducibility of findings in forensic and clinical investigations.

Although advances in immunohistochemical techniques offer new opportunities, practical application requires a more robust body of scientific evidence. Future research should therefore aim to fill identified gaps by providing reliable and scientifically validated diagnostic tools to improve accuracy and reproducibility in forensic investigations.

## Conclusion

In conclusion, while recent advancements in immunohistochemical techniques offer opportunities for enhancing the diagnosis of hypothermia as a cause of death, the existing evidence remains fragmented and lacks robust statistical validation. The variability and limited reproducibility across studies underscore the need for more comprehensive and methodologically rigorous research to develop reliable diagnostic criteria. Without further investigations, the practical use of these immunohistochemical markers in forensic pathology remains uncertain. Ongoing efforts to refine diagnostics are crucial to ensure scientifically validated and reliable forensic evaluations.

## Key points

1. Hypothermia is the decrease in core body temperature to below 35 °C;
2. macroscopic observations in cases of fatal hypothermia include Wischnewsky spots, erythema, and hemorrhaging in the iliopsoas muscles;
3. immunohistochemistry has been considered a potentially useful tool in hypothermia diagnosis;
4. the diagnosis of death by hypothermia may hold significant value in legal proceedings. Therefore, highly reliable, scientifically validated diagnostic tools are needed to present strong evidence before a judge;

### Abbreviations

| | |
|---|---|
| PRISMA | Preferred Reporting Items for Systematic Reviews and Meta-Analyses |
| ACTH | Adrenocorticotropic Hormone |
| Hsp70 | Heat Shock Protein 70 |
| Hsp27 | Heat Shock Protein 27 |
| HSF1 | Phosphorylated Heat Shock Factor 1 |
| CgA | Chromogranin A |
| TSH | Thyroid-Stimulating Hormone |
| AQP | 1-Aquaporin 1 |
| AQP | 5-Aquaporin 5 |
| ANP | Atrial Natriuretic Peptide |
| BNP | Brain Natriuretic Peptide |
| mRNA | Messenger Ribonucleic Acid |
| C5b9 | Membrane Attack Complex C5b-9 |
| CLDN | 5-Claudin-5 |
| GFAP | Glial Fibrillary Acidic Protein |
| Hb | Hemoglobin |
| ICAM | 1-Intercellular Adhesion Molecule 1 |
| IL | 1β-Interleukin-1 Beta |
| IL | 10-Interleukin-10 |
| MMP | 2-Matrix Metallopeptidase 2 |
| MMP | 9-Matrix Metallopeptidase 9 |
| MAP2 | Microtubule-Associated Protein 2 |
| SP | A-Lung Surfactant A |
| SP | D-Lung Surfactant D |
| SIRT1 | Sirtuin 1 |
| TNF | α-Tumor Necrosis Factor Alpha |
| TM | Thrombomodulin |
| Ub | Ubiquitin |
| GH | Growth Hormone |
| bFGF | Basic Fibroblast Growth Factor |
| ROC | Receiver Operating Characteristic |
| ANOVA | Analysis of Variance |
| CSF | Cerebrospinal Fluid |

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12024-024-00934-0.

## Declarations

**Conflicts of interest** The authors declare no conflicts of interest.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

## References

1. Turk EE, Hypothermia. Forensic Sci Med Pathol. 2010;6(2):106–15.
2. Davis PR, Byers M. Accidental hypothermia. J R Army Med Corps. 2005;151(4):223–33.
3. Duguid H, Simpson RG, Stowers JM. Accidental hypothermia. Lancet Lond Engl. 1961;2(7214):1213–9.
4. Mair P, Kornberger E, Furtwaengler W, Balogh D, Antretter H. Prognostic markers in patients with severe accidental hypothermia and cardiocirculatory arrest. Resuscitation. 1994;27(1):47–54.
5. Vretenar DF, Urschel JD, Parrott JC, Unruh HW. Cardiopulmonary bypass resuscitation for accidental hypothermia. Ann Thorac Surg. 1994;58(3):895–8.
6. Doberentz E, Markwerth P, Wagner R, Madea B. Expression of Hsp27 and Hsp70 and vacuolization in the pituitary glands in cases of fatal hypothermia. Forensic Sci Med Pathol. 2017;13(3):312–6.
7. Yang C, Sugimoto K, Murata Y, Hirata Y, Kamakura Y, Koyama Y, et al. Molecular mechanisms of Wischnewski spot development on gastric mucosa in fatal hypothermia: an experimental study in rats. Sci Rep. 2020;10(1):1877.
8. Doberentz E, Markwerth P, Madea B. Fatty degeneration in renal tissue in cases of fatal accidental hypothermia. Romanian J Leg Med. 2017;25:152–7.
9. Knight B, Saukko P. Knight's Forensic Pathology. In: IV. pp. 415–24.
10. Mant AK. Autopsy diagnosis of accidental hypothermia. J Forensic Med. 1969;16(4):126–9.
11. Hirvonen J. Necropsy findings in fatal hypothermia cases. Forensic Sci. 1976;8(2):155–64.
12. Madea B, Preuss-Wössner J, Lignitz E. Morphological findings in fatal hypothermia and their pathogenesis.
13. Natanti A, Mazzanti R, Palpacelli M, Turchi C, Tagliabracci A, Pesaresi M. Death following extreme temperature exposure: histological, biochemical and immunohistochemical markers. Med Sci Law. 2021;61(1suppl):36–41.
14. Yoshida C, Ishikawa T, Michiue T, Quan L, Maeda H. Postmortem biochemistry and immunohistochemistry of chromogranin A as a stress marker with special regard to fatal hypothermia and hyperthermia. Int J Legal Med. 2011;125(1):11–20.
15. Yoshida C, Ishikawa T, Michiue T, Zhao D, Komatsu A, Quan L, et al. Immunohistochemical distribution of chromogranin A in medicolegal autopsy materials. Leg Med Tokyo Jpn. 2009;11(Suppl 1):S231–233.
16. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan—a web and mobile app for systematic reviews. Syst Rev. 2016;5:210.
17. Rayyan– Intelligent Systematic Review - Rayyan [Internet]. 2021 [cited 2024 Jun 22]. https://www.rayyan.ai/
18. Pakanen L, Kaija H, Kortelainen ML, Särkioja T, Porvari K. Victims of lethal hypothermia have decreased levels of thrombomodulin in myocardium and urine. Int J Legal Med. 2015;129(2):289–96.
19. Ishikawa T, Michiue T, Zhao D, Komatsu A, Azuma Y, Quan L, et al. Evaluation of postmortem serum and cerebrospinal fluid levels of thyroid-stimulating hormone with special regard to fatal hypothermia. Leg Med Tokyo Jpn. 2009;11(Suppl 1):S228–230.
20. Sakurada M, Asano M, Takahashi M, Kuse A, Morichika M, Nakagawa K, et al. Estimates of exposure to cold before death from immunohistochemical expression patterns of HSP70 in glomerular podocytes. Int J Legal Med. 2013;127(4):783–90.
21. Wang Q, Ishikawa T, Michiue T, Zhu BL, Guan DW, Maeda H. Evaluation of human brain damage in fatalities due to extreme environmental temperature by quantification of basic fibroblast growth factor (bFGF), glial fibrillary acidic protein (GFAP), S100β and single-stranded DNA (ssDNA) immunoreactivities. Forensic Sci Int. 2012;219(1–3):259–64.
22. Ishikawa T, Quan L, Li DR, Zhao D, Michiue T, Hamel M, et al. Postmortem biochemistry and immunohistochemistry of adrenocorticotropic hormone with special regard to fatal hypothermia. Forensic Sci Int. 2008;179(2–3):147–51.
23. Palmiere C, Teresiński G, Hejna P. Postmortem diagnosis of hypothermia. Int J Legal Med. 2014;128(4):607–14.
24. Lim C, Duflou J. Hypothermia fatalities in a temperate climate: Sydney, Australia. Pathol (Phila). 2008;40(1):46–51.
25. Ishikawa J, Oshima M, Iwasaki F, Suzuki R, Park J, Nakao K, et al. Hypothermic temperature effects on organ survival and restoration. Sci Rep. 2015;5:9563.
26. Torlakovic EE. Fit-for-purpose immunohistochemical biomarkers. Endocr Pathol. 2018;29(2):199–205.
27. Ishikawa T, Miyaishi S, Tachibana T, Ishizu H, Zhu BL, Maeda H. Fatal hypothermia related vacuolation of hormone-producing cells in the anterior pituitary. Leg Med Tokyo Jpn. 2004;6(3):157–63.
28. Lesnikova I, Schreckenbach MN, Kristensen MP, Papanikolaou LL, Hamilton-Dutoit S. Usability of immunohistochemistry in forensic samples with varying decomposition. Am J Forensic Med Pathol. 2018;39(3):185–91.
29. Baudier J, Gentil BJ. The S100B protein and partners in adipocyte response to cold stress and adaptive thermogenesis: facts, hypotheses, and perspectives. Biomolecules. 2020;10(6):843.
30. Usman MG, Rafii MY, Martini MY, Yusuff OA, Ismail MR, Miah G. Molecular analysis of Hsp70 mechanisms in plants and their function in response to stress. Biotechnol Genet Eng Rev. 2017;33(1):26–39.
31. Yang C, Wang Z, Kang Y, Yi Q, Wang T, Bai Y, et al. Stress granule homeostasis is modulated by TRIM21-mediated ubiquitination of G3BP1 and autophagy-dependent elimination of stress granules. Autophagy. 2023;19(7):1934–51.
32. Hleșcu AA, Grigoraș A, Covatariu G, Moscalu M, Amalinei C. The Value of Myocardium and kidney histopathological and immunohistochemical findings in Accidental Hypothermia-related fatalities. Med Kaunas Lith. 2022;58(11):1507.
33. Trautz F, Dreßler J, Stassart R, Müller W, Ondruschka B. Proposals for best-quality immunohistochemical staining of paraffin-embedded brain tissue slides in forensics. Int J Legal Med. 2018;132(4):1103–9.
34. Chen XS, Chu J, Yang LJ, Wang T, Tao LY. Application of immunohistochemistry and special staining technique in Forensic Traumatic Pathology Identification. Fa Yi Xue Za Zhi. 2021;37(5):666–72.

35. Miller RT. Avoiding pitfalls in diagnostic immunohistochemistry-important technical aspects that every pathologist should know. Semin Diagn Pathol. 2019;36(5):312–35.
36. Chen JH, Michiue T, Ishikawa T, Maeda H. Molecular pathology of natriuretic peptides in the myocardium with special regard to fatal intoxication, hypothermia, and hyperthermia. Int J Legal Med. 2012;126(5):747–56.
37. Miyazato T, Ishikawa T, Michiue T, Maeda H. Molecular pathology of pulmonary surfactants and cytokines in drowning compared with other asphyxiation and fatal hypothermia. Int J Legal Med. 2012;126(4):581–7.
38. Ishikawa T, Yoshida C, Michiue T, Perdekamp MG, Pollak S, Maeda H. Immunohistochemistry of catecholamines in the hypothalamic-pituitary-adrenal system with special regard to fatal hypothermia and hyperthermia. Leg Med Tokyo Jpn. 2010;12(3):121–7.
39. Wang Q, Ishikawa T, Michiue T, Zhu BL, Guan DW, Maeda H. Molecular pathology of pulmonary edema in forensic autopsy cases with special regard to fatal hyperthermia and hypothermia. Forensic Sci Int. 2013;228(1–3):137–41.
40. Tsokos M, Rothschild MA, Madea B, Rie M, Sperhake JP. Histological and immunohistochemical study of Wischnewsky spots in fatal hypothermia. Am J Forensic Med Pathol. 2006;27(1):70–4.
41. Doberentz E, Preuss-Wössner J, Kuchelmeister K, Madea B. Histological examination of the pituitary glands in cases of fatal hypothermia. Forensic Sci Int. 2011;207(1–3):46–9.
42. Preuss J, Dettmeyer R, Poster S, Lignitz E, Madea B. The expression of heat shock protein 70 in kidneys in cases of death due to hypothermia. Forensic Sci Int. 2008;176(2–3):248–52.
43. Shimizu K, Ohtani S, Shiono H, Fukusima T, Sasaki M. Expression of ubiquitin protein in each organ at death from hypothermia. Forensic Sci Int. 1997;86(1–2):61–8.
44. Kitamura O, Gotohda T, Ishigami A, Tokunaga I, Kubo S, ichi, Nakasono I. Effect of hypothermia on postmortem alterations in MAP2 immunostaining in the human hippocampus. Leg Med. 2005;7(1):24–30.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.