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Postmortem bone marrow analysis in forensic science: Study of 73 cases and review of the literature

Lucia Tattoli [a], Michael Tsokos [b], Julia Sautter [b], Joannis Anagnostopoulos [c], Eloisa Maselli [a], Giuseppe Ingravallo [d], Mario Delia [e], Biagio Solarino [a,*]

[a] Institute of Legal Medicine, University of Bari, P.zza G. Cesare, 11, 70124 Bari, Italy
[b] Institute of Legal Medicine and Forensic Sciences, University Medical Centre Charité, University of Berlin, Turmstr. 21, Building N, 10559 Berlin, Germany
[c] Institute of Pathology, University Medical Centre Charité, University of Berlin, Turmstr. 21, Building N, 10559 Berlin, Germany
[d] Department of Pathological Anatomy, University of Bari, P.zza G. Cesare, 11, 70124 Bari, Italy
[e] Department of Pathology and Hematology, University of Bari, P.zza G. Cesare, 11, 70124, Bari, Italy

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ABSTRACT

In forensic sciences, bone marrow (BM) is an alternative matrix in postmortem toxicology because of its good resistance to autolysis and contaminations. Nevertheless, few studies have been focused on postmortem BM morphological changes after pathological stimuli. We examined 73 BM samples from forensic autopsies; causes of death were both natural and traumatic. BM samples were collected from the sternum by needle aspiration and biopsy; in selected cases, immunohistochemistry was performed. Few autolytic changes were found; BM cellularity decreased with increasing age and postmortem interval. Notable cell changes were detected in 45 cases (61.64%): neoplastic ($n = 4$), and non-neoplastic BM findings ($n = 41$), including multiorgan failure/sepsis ($n = 26$), myelodisplastic-like conditions ($n = 11$), and anaphylactic reactions ($n = 4$). The results showed that BM cellularity supported circumstantial and autopsy findings, suggesting that BM samples could be a useful tool in forensic science applications.

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1. Introduction

In clinical practice, analysis of bone marrow (BM) is a useful technique, especially in the diagnosis of several haematological disorders such as anaemia, leukaemia, and myeloma [1–8]. It is also effective in the diagnosis of non hematological diseases, to detect metastatic spread, storage disorders, in the staging of solid tumors, in performing microbiological culture in patients with unexplained fever, HIV infection, and in the follow-up of patients after chemotherapy or transplantation [2,4,9–15].

In the forensic sciences field, most of the studies published in recent years have described the use of BM as an alternative tissue in toxicology analyses, to detect alcohol and drug content both in humans [16–21] and in laboratory animals [22–26], and in cases when blood samples are unavailable such as skeletonized, bloodless or putrefied cadavers [16,17,19,22,27–32]. BM is also used in forensic genetic analysis [33], and in the diagnosis of drowning using the diatom test [34] or to detect aquatic bacteria [35].

In the forensic literature, BM has been collected from several bones, according to availability, e.g. the iliac crest [29], pelvic bone, vertebrae (by puncturing) [20], femur (by section of the cortical bone), sternum, and ribs (by compressing the cut ends) [16,18,36]. Nevertheless, a standard sampling procedure has not yet been established in the scientific community. The importance of this analysis was stressed by Grellner et al. [37], in a retrospective report of 46 exhumations that demonstrated BM histological preservation after 3 months. The possibility of contamination is also reduced [17], because of the physical barrier provided by cortical bone against postmortem exogenous contamination, exposure and damage (trauma, animal action) [29,33]. Furthermore, in an analysis of 225 BM samples from autopsies, Roll et al. [38] observed little deterioration of the anatomical structure of these specimens in comparison with the alterations seen in other organ samples.

BM has a key role in the regulation of systemic homeostasis through hematopoiesis [11,12,39–41], involving cell-to-cell interactions and appropriate chemical signals (cytokines) [39,42–44]. Hematopoietic tissue is sensitive to external influences, such as

* Corresponding author. Tel.: +39 80 5594029; fax: +39 80 5721099.
E-mail address: biagiosolarino@libero.it (B. Solarino).

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dietary restriction, malnutrition, chronic inflammation, toxicity, and proliferative or neoplastic disorders [39,45,46]. Cell differentiation also occurs as a result of several stimuli such as infections or other systemic diseases. Cell morphology analyses can demonstrate such changes.

The present study was aimed at defining a possible supporting role of postmortem BM analysis in forensic pathology and gaining a better understanding of BM morphological features in several pathological conditions that might even have been unknown during the patient's lifetime, thus collecting a more complete documentation of the cause of death.

2. Materials and methods

The BM samples were collected from cases ($n = 73$) of medicolegal autopsy performed at the Department of Legal Medicine in Bari (Italy) and at the Institute of Legal Medicine and Forensic Sciences in Berlin (Germany). All the cadavers were preserved and kept cool.

The age of subjects ranged from 2 to 91 years; there were 26 females and 47 males. Subjects died of various different causes: natural (48 cases), or traumatic (25 cases), as indicated in Table 1. Exclusion criteria were fractures of the sternum or ribs closed at the cartilaginous part, to prevent interference with the results due to bleeding in the samples (as a consequence of fractures).

The BM samples were taken from 5 h up to a maximum of 198 h postmortem, with the exception of one subject found dead after 1 month, whose BM biopsy was performed at autopsy two days later; the BM aspirate did not yield any tissue.

During the necropsies, after routine removal of the sternum by cutting the ribs at the cartilaginous part, BM was sampled from the first part of the sternum, in the midportion, by both needle aspiration and biopsy. BM aspirate (2–5 ml) was collected in a 10 ml syringe connected to a BM needle (Jamshidi 11G, Baxter Healthcare Corp., IL) by puncturing the sternum. About 0.25 to 0.5 ml of aspirate were delivered onto clear glass slides and smears were prepared. Films were spread immediately without adding any anticoagulant. When dry, the films were stained with May-Grünwald-Giemsa stain, then examined (preferably using an immersion oil, 100× lens in a light microscope). BM trephine biopsies were carried out on the same portion of sternum by biopsy needle (Jamshidi 10 × 8 cm—Cardinal Health, USA), cutting at least 2 cm into the depth of the bone to avoid unrepresentative probes. In cooperation with Department of Pathology, the specimens were fixed in 4% neutral buffered formalin solution for about 24 h. Subsequently, the samples were decalcified, paraffin-embedded, cut into 5–6 mm slices and routinely stained with Hematoxylin and Eosin (H&E).

In selected cases, immunohistochemical analyses were performed. After standard antigen retrieval procedures, the sections of paraffin-embedded BM were incubated overnight at 4 °C with primary polyclonal rabbit anti-human Myleoperoxidase (anti-MPO) at a dilution of 1:16,000 (source: Dako, Glostrup, Denmark).

3. Results

All the BM biopsies were evaluable. The aspirates were suitable in 91.7% of cases, because a "dry tap" occurred in six cases, mostly age-correlated.

3.1. BM cellularity and aging

In all specimens, the age-related involution of BM was evident, featuring a loss of hematopoietic tissue and a corresponding increase in the amount of adipose tissue, filling the non-hematopoietic marrow space. These findings were more evident in biopsies than in aspirates. In adult subjects (especially aged >65 years) hypocellular BM was observed; this evidence was correlated to the normal aging process, and consisted predominantly of mature fat cells with an increased number of fibroblasts and collagen fibers. In young subjects (up to the age of 30) we observed normocellular BM, while the overall number of capillaries and sinusoids was higher in infancy than in adulthood.

3.2. BM cellularity and postmortem interval (PMI)

The anatomical structure of BM samples showed less autolytic changes than those in other organs (e.g. lung, liver, kidney, brain). The lineage precursors were still recognizable even if the cellularity decreased with the PMI, being the time elapsed between death and the sampling. Such changes were gradual and more evident in BM biopsies (Fig. 1a–d). Erythroid precursors underwent the most evident autolytic changes in comparison with other lineages, appearing as pale cells in coagulative necrosis.

In BM aspirates (Fig. 2a–c) tissue autolysis was also related to the increasing PMI: nuclei gradually shrank and darkened and the cells tended to coalesce; vacuolar degeneration of the cell cytoplasm, which became eosinophilic, was evident, and the granulocytic cells underwent necrotic modifications of their granules, which were no longer localized.

Fig. 3 shows the preserved BM structure of a drowned subject, whose body was found 1 month after death; the trabecular structure is well recognizable, and some cells (mast cell precursors, erythroid cells) are still observable, even though many other necrotic pale cells are present.

3.2.1. BM cellularity and pathological status

Important changes in BM cellularity were detected in 45 cases (61.64%).

Neoplastic findings were found in four cases, including known diagnoses of chronic myelomonocytic leukemia ($n = 1$) and lymphoblastic leukemia ($n = 1$), and two cases of metastatic carcinoma that had not been diagnosed during the subject's lifetime, both originating from gastric cancer. One of these was a 35-year-old male with a medical history of gastric cancer and liver metastasis who died in hospital, after complications while undergoing biopsy of a hepatic nodule. At the autopsy, performed to evaluate any liability for medical malpractice, the stomach and liver tumors were confirmed. The sternal BM biopsy (Fig. 4)

Table 1
Causes of death.

Cause of death	N	Male/female	Age (yrs) range	Postmortem interval (h) range
Natural deaths	48			
Multiorgan failure[a]	19	9/10	54–91	24–198
Acute cardiovascular diseases[b]	18	12/6	39–87	5–170
Respiratory failure (pneumonia)	7	3/4	65–85	24–70
Anaphylaxis (drug)	4	2/2	22–41	24–72
Traumatic deaths	*25*			
Intoxication (heroin, cocaine, methadone)	8	8/0	25–35	24–84
Burns	7	5/2	48–72	24–72
Haemorrhagic shock (gunshot wound, stabbing, road accident)	6	5/1	22–63	48–120
Asphyxia (suffocation, hanging, drowning)	4	3/1	2–41	24–95[c]

[a] MOF in patients with no traumatic injuries (cancer, intestinal ischemia, peritonitis, abdominal surgery).
[b] Acute cardiovascular diseases included malignant arrhythmias, cardiac sudden death, acute myocardial infarction, ruptured aneurysms.
[c] 24–95 h, except a case with a PMI of 1 month.

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