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REVIEW

Microbiology in minimally invasive autopsy: best techniques to detect infection. ESGFOR (ESCMID study group of forensic and post-mortem microbiology) guidelines

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Abstract

This manuscript aims to: 1) provide specific guidelines on PMM techniques in the setting of minimally invasive autopsy (MIA), both for pathologists collecting samples and for microbiologists advising pathologists and interpreting the results and 2) introduce standardization in PMM sampling at MIA. Post-mortem microbiology (PMM) is crucial to identify the causative organism in deaths due to infection. MIA including the use of post-mortem (PM) computed tomography (CT) and PM magnetic resonance imaging (MRI), is increasingly carried out as a complement or replacement for the traditional PM. In this setting, mirroring the traditional autopsy, PMM aims to: detect infectious organisms causing sudden unexpected deaths; confirm clinically suspected but unproven infection; evaluate the efficacy of antimicrobial therapy; identify emergent pathogens; and recognize medical diagnostic errors. Meaningful interpretation of PMM results requires careful evaluation in the context of the clinical history, macroscopic and microscopic findings.These guidelines were developed by a multidisciplinary team with experts in various fields of microbiology and pathology on behalf of the ESGFOR (ESCMID – European Society of Clinical Microbiology and Infectious Diseases - Study Group of Forensic and Post-mortem Microbiology, in collaboration with the ESP -European Society of Pathology-) based on a literature search and the author's expertise. Microbiological sampling methods for MIA are presented for various scenarios: adults, children, developed and developing countries. Concordance between MIA and conventional invasive autopsy is substantial for children and adults and moderate for neonates and maternal deaths. Networking and closer collaboration among microbiologists and pathologists
Keywords Traditional autopsy · Forensic sampling · Infection · Forensic microbiology · Minimally invasive autopsy · Post-mortem microbiology

Introduction

Post-mortem microbiology (PMM) is crucial to identify the causative organism in deaths due to infection. Although the traditional autopsy remains the gold standard procedure to obtain samples for PMM, the lack of trained human resources, and other constraints such as cultural and religious requirements, have led to the introduction of minimally invasive autopsy (MIA) techniques as an alternative [1]. In some cases, MIA includes the procurement of tissue

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samples, also known as Minimally Invasive Tissue Sampling (MITS), using fine needles to collect small amounts of tissue from key organs and body fluids. Currently, MIA is increasingly carried out as a complement or replacement for the traditional complete autopsy, mostly in low- and middle-income countries (LMIC).

The dead human body is a complex microbial ecological system that varies significantly between individuals as a result of diet, lifestyle and geographical factors [2, 3]. It is therefore important to highlight that a positive result from PMM may reflect a genuine infection but may also reflect sample contamination, commensal organisms and/or post-mortem bacterial translocation (PMBT). The incidence of post-mortem contamination and PMBT has been estimated to approximate 20% in traditional autopsies where PMM has been conducted [4, 5]. However, when standardized procedures are adhered to, post-mortem contamination can be kept below 10% [6].

PMBT is a natural phenomenon in which endogenous commensal gut bacteria, most commonly *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus spp.,* Clostridia, and streptococci multiply and migrate into the blood and tissues. PMBT is a driver of putrefactive decomposition, which begins approximately four minutes after death [2, 7–9]. Although PMBT begins within eight hours of death, it is not solely dependent on the post-mortem interval (PMI). Agonal spread of microorganisms throughout the body during the process of dying and resuscitation has been suggested but is not universally accepted [6, 10, 11]. Aerobic bacteria proliferate early during the putrefactive process, to be overtaken later by anaerobes [2].

Sample contamination during collection of peripheral blood is most commonly due to the introduction of coagulase-negative staphylococci from the skin [10]. In general, a mixed bacterial growth likely represents sample contamination whilst single isolates are generally interpreted to represent true positives [6, 11, 12]. Despite close adherence to sampling protocols, a pure growth of a single microorganism from multiple sites may still represent contamination rather than infection [4, 10]. Yet isolation of a pathogenic organism from multiple sites at autopsy most likely represents a true ante-mortem bacteremia [4].

A prolonged PMI and putrefaction are usually perceived to impair the yield of PMM [13]. Tuomisto et al. [14], using real-time quantitative PCR to investigate bacterial migration from the gut into the blood, liver, portal vein, mesenteric lymph node, and pericardial fluid of cadavers stored at 4°C, were able to demonstrate that the relative amounts of intestinal bacterial DNA (bifidobacteria, *Bacteroides*, *Enterobacter* and Clostridia) increased with time. However, a prolonged PMI does not uniformly increase the risk of PMBT or the yield in obtaining positive post-mortem cultures [6, 15]. Differences in detection techniques (routine bacteriology cultures versus molecular techniques) could be part of the explanation in contradictory study results.

Palmiere et al. [4] showed that bacterial cultures and real-time polymerase chain reaction (qPCR) analyses were reliable, not yielding false-positive results in the detection of *Neisseria meningitidis*, *Listeria monocytogenes*, *Streptococcus pneumoniae* or *Haemophilus influenzae* in CSF obtained from the lateral ventricles and cisterna magna in severely decomposing adults known to have not died infectious deaths. Positive results were obtained in both bacterial culture and qPCR analysis of CSF from decomposing controls with known meningitis. Also, reliable results were obtained in other *N. meningitis* qPCR validation studies performed in post-mortem samples [16].

Overall, PMBT is a frustrating "side-effect" of death. From a practical point of view, refrigeration of the body minimizes this phenomenon [6]. There is increasing interest in the possibility that exploration of successional changes in the human microbiome after death using metagenomics may have a role to play in determining the PMI [3, 8], particularly in decomposing bodies [2, 17, 18].

As PMM is not yet an extended practice in MIA, the contaminating effects of PMBT have not been evaluated yet. In the following paragraphs, we develop in more detail microbiological sampling in MIA. With careful interpretation, PMM is a useful tool to identify correctly an infectious cause of death (COD). Our study aims to provide useful guidelines on PMM sampling techniques in MIA, both to the pathologist requesting the analysis and to the microbiologist advising the pathologist and interpreting the results.

General post-mortem microbiology sampling technique

PMM samples should be collected as soon as possible and ideally within 24 hours of death [19], but it is often impossible to perform MIA in such a short time frame, especially in poor settings. The collection of samples for PMM can still be fruitful [6], however the PMI should always be considered along with the imaging and/or macroscopic and microscopic features of the organs when interpreting the results (See Table 1).

The nature of the samples to be collected varies depending on the clinical history. Specific examples are considered below. In infant and childhood death without symptoms, a standard set of samples is recommended in the traditional PM: nasopharyngeal swab, CSF, blood, lung, spleen, heart, and bowel content. If sepsis, bacteremia or other conditions are suspected, it is recommended to add samples from other tissues (Table 1). [19–21]. According to

the method of MIA used, the aim would be to replicate these samples as much as possible. Some centers complement the traditional full autopsy or the MIA MSCT (Multi-Slice-Computed Tomography), MRI (Magnetic Resonance Imaging), using MITS (tissue sampling through fine needle) or laparoscopic/thoracoscopic approaches which allow direct visualization and sampling of organs [22, 23].

A variety of other conditions may cause systemic inflammatory response syndrome and mimic sepsis in the absence of infection [24]. According to the context of the MIA, the provision of non-microbiological samples, such as those referred for toxicological screening, metabolic analysis and histopathological examination may be required.

Where samples are obtained by percutaneous needle puncture/aspiration in MITS, the body must first be appropriately positioned, and the skin should be cleaned with sterile water and disinfected either with alcohol-based solution containing chlorhexidine or iodine. Isopropyl alcohol should only be used after samples for toxicological analysis have been obtained (or the toxicologist must be notified of its use) [19]. Prior post-mortem angiography via femoral cannulation does not impede the collection of peripheral blood for PMM provided that the skin is disinfected prior to cannulation for angiography [5].

Ideally, a separate set of sterile instruments should be used to collect each sample, in order to avoid cross-contamination [13].

Tissue samples for microbiology should be stored in sterile containers or bottles without additives, refrigerated until transport and sent to the microbiology laboratory within 24-48 hours after the autopsy [13, 18]. It is recommended to cryopreserve additional samples at -80ºC for future molecular analyses in the event they are required.

Consideration must be given to the health and safety of mortuary workers during the collection of PMM samples, as the decedent's microbiome may pose a risk. Specific measures to protect mortuary staff from potential infectious hazards have been described elsewhere [13, 25]. Direct contact and puncture wounds are the most important routes for transmission of blood-borne infections, and aerosol transmission is mainly a risk for airborne pathogens such as *Mycobacterium tuberculosis* complex or the MERS, SARS or COVID-19 coronaviruses [26]. Universal safety precautions to be applied are personal protective equipment including eye protection, a surgical mask, surgical gown, waterproof apron, gauntlets, surgical gloves and, optionally, a cut-resistant glove on the non-dominant hand. However, when airborne pathogens are anticipated, additional respiratory protection, such as a free flight phase two (FFP2) or even FFP3 masks for highly contagious pathogens such as COVID-19 is required [27]. Where fungal growth is evident macroscopically either during the crime scene review, exhumation or at autopsy, respiratory protection should also be worn due to the risk of inhaling spores from *Aspergillus fumigatus* complex and *Candida albicans* [18].

Different settings for microbiology in MIA

The adult MIA

Changing societal, cultural, religious and political attitudes around the world have seen the conventional invasive autopsy become less acceptable and less frequent [1, 28, 29]. MIA has gained acceptability and is a valid alternative to the traditional autopsy in the investigation of natural adult deaths, especially in low-income countries [28, 30–33].

As in the conventional PM, MIA protocol includes a review of the available history of events leading to death and a thorough external examination. Unlike the conventional complete autopsy, in MIA the body cavities are not opened. Instead, key organs may be sampled through percutaneous puncture using specific needles (see below under MIA in developing countries). Swabs represent another alternative to be used in noninvasive post mortems. Where facilities and resources permit, non-invasive or MIAs using either multi-slice computed tomography (MSCT) or MRI scans with or without angiography may be used to replace or limit the conventional autopsy [31, 34, 35]. In adults, MSCT has been shown to be superior to MRI in detecting the COD [34]. The combination of post-mortem whole body MSCT scan, CT angiography and tissue biopsy has been shown to be the most sensitive technique in detecting the COD when MIAs are undertaken and surpass non-invasive techniques alone [35].

Microbiology sampling during the adult MIA

While MIA can reduce the need to undertake invasive autopsies in patients with known or suspected Hazard Group 3 infections such as HIV or COVID-19 [31, 35–37], very few recent articles detail the collection of samples for microbiological examination in adults during MIA. Previous studies have focused on the collection of biopsies for histopathological examination [38, 39]. In some studies, patients with known high-risk infections have been actively excluded and/or tissue sampling limited to histopathological examination [30, 40]. Previous research has focused on the role of MIA to assess the prevalence of tuberculosis and other infections in patients with known HIV infection [41]. Collection of blood, CSF and samples from the lungs, liver and at MIA conducted 3-6 days after death have demonstrated that tuberculosis and other bacterial infections are the commonest COD

Table 1 Macroscopic and imaging features in infectious conditions

Condition	Macroscopic appearance	Imaging*	Etiological agents	Remarks and references
Respiratory infections				
Tonsillitis, laryngitis, tracheitis, bronchitis	Congested mucosa +/- mucopurulent secretions	*Radiographs:* Enlarged tonsils *CT:* Enlarged "kissing" tonsils, parapharyngeal fat stranding *MRI:* Enlarged tonsils, low to isointense on T1, high signal on T2	Bacteria Viruses	
Pneumonia	-Heterogeneous, multifocal affecting several lobes (bronchopneumonia) or -a homogenous consolidation affecting one or more lobes (lobar pneumonia) 4 histopathological stages: congestive phase: enlarged and congested lung – red hepatisation: a reddish firm and consolidated lung – grey hepatisation: an opaque lung with grey discoloration and a purulent exudate on the cut surface - resolution	Air space shadowing with air bronchograms in a rounded (rounded pneumonia), lobar (lobar pneumonia) or patchy (bronchopneumonia) distribution	Bacteria Viruses Fungi	Clinical classification: community acquired Hospital acquired Ventilator associated Aspiration pneumonia More information on etiological agents in [21]
Tuberculosis	-Disseminated Tuberculosis: multiple small whitish/yellowish, well circumscribed, firm nodules +/- a central caseous necrosis. -miliary tuberculosis: numerous whitish nodules (2mm diameter) invariably involving the pleural surface, may also be present in vertebra and other organs	Perihilar/paratracheal lymphadenopathy (primary MTB) Calcification of Ghon focus and lymph nodes plus solitary or multiple granulomas (latent MTB) Consolidation, obstructive atelectasis, cavitation (progressive primary MTB) Innumerable small (≤2mm) calcified nodules best seen on CT (military MTB) Pleural/pericardial thickening and effusion (pleural/pericardial MTB)	*Mycobacterium tuberculosis* complex (MTB)	
Abscess	A cavity filled with necrotic and purulent material.	Rounded cavity with air/fluid level. There may be surrounding consolidation	Bacteria	May be associated to an adjacent area of bronchopneumonia
Empyema thoracis	A fibrinopurulent exudate on the pleural surfaces or frank pus in the pleural cavity.	*Radiographs:* Lenticular-shaped fluid density in the pleural space *CT:* May show locules of gas. Displacement and compression of adjacent lung. "Split pleura" sign		A possible complication of lobar bronchopneumonia or pneumonia, lymphatic or vascular dissemination of a distant infection, or from a hepatic or subphrenic abscess

Table 1 (continued)

Condition	Macroscopic appearance	Imaging*	Etiological agents	Remarks and references
COVID-19	Pleurisy, pericarditis, lung consolidation and pulmonary oedema. Possibility of lung weight increased and also of purulent inflammation due bacterial co-infection	*Radiographs:* multiple ground glass opacities *CT:* GGO, mixed GGO, consolidation, reticulation. [69]	SARS-CoV-2	Although mainly affecting the lung (diffuse alveolar damage, hyaline membranes, macrophages and CD4+ T cell lymphocytes, and microthrombus) other organs can also be damaged: heart (myocarditis), spleen (decreased numbers of lymphocyte, cell degeneration and necrosis), vessels, liver, kidney and others, which should be sampled in the MIA [70, 71],
Central nervous system infections				
Meningo-encephalitis	Non-specific aspect of brain: pale, swollen, with flattened gyri and devoid of any exudate	Unenhanced post-mortem imaging may be normal *CT:* Mild hydrocephalus. Hyperdensity surrounding basal cisterns *MRI:* Sulci less hypointense than normal (T1), hyperintense signal in sulci (FLAIR)	Viruses	More information on etiological agents in [21]
	N. meningitidis: no exudate. Other bacteria: a purulent exudate, regularly associated with middle ear infection (eg. *S. pneumoniae*)		Bacteria	Post-mortem culture often negative for *N. meningitidis* since fastidious organism. PCR from skin petechiae or CSF gives highest yield. Swab of the middle ear is important in PMM [63–68, 72, 73]
	- Dense gelatinous inflammatory exudate, mainly in the basal cisterns - meningeal or parenchymatous location - release of *M. tuberculosis* complex and dissemination and causing encephalitis		*Mycobacterium tuberculosis* complex	
			Candida spp., Mucorales spp., *Aspergillus fumigatus* complex, *Cryptococcus* spp.	Most often in immunocompromised patients

Table 1 (continued)

Condition	Macroscopic appearance	Imaging*	Etiological agents	Remarks and references
Cerebral phaeohyphomycosis		Single or multiple brain abscesses seen on CT or MRI as solid round/oval space occupying lesions with central necrosis/fluid and perilesional edema. Central diffusion restriction on MRI highly suggestive of an abscess rather than tumour	*Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Cladophialophora bantiana* [74]	Healthy individuals endemic regions contact and airborne precautions
Cerebral abscess in the deep grey matter and in the cortex		Solid round/oval space occupying lesion with central necrosis/fluid and perilesional edema. Central diffusion restriction on MRI highly suggestive of an abscess rather than tumour	Parasites (eg. Toxoplasmosis)	Immunocompromised patients
Cardiovascular infection				
Endocarditis	Friable vegetations containing fibrin, cellular debris, bacteria. Vegetations may erode the adjacent valve ring and cause annular abscesses	*Radiographs/CT:* Non-specific, patchy consolidation, cardiomegaly with pericardial effusion, pleural effusion (on occasion) *MRI:* May show valvular vegetations	*Streptococcus viridans*, *Staphylococcus aureus*, *Enterococcus* spp., *Streptococcus gallolyticus*, HACEK group bacteria*, atypical mycobacteria	- In children with repaired complex heart diseases: mean age of 12.3+/-5.5 yrs, viridans streptococci and *S. aureus* [75] - *S. aureus* most prevalent in IV drug users - *M. chimaera* endocarditis reports after open heart surgery linked to contamination of heater cooler units [76, 77]
Myocarditis	Heart is floppy and dilated. Myocardium may appear macroscopically normal, although frequently pale with punctate haemorrhagic areas.	Non-specific post-mortem imaging findings	Viruses: Enteroviruses, *Parvovirus B19*, *Human herpesvirus 6 and 8*, Adenoviruses; *Trypanosoma cruzi*** [78]	Human Immunodeficiency virus, *M. tuberculosis* complex may cause myocarditis [79] More information on etiological agents in [20, 21]
Pericarditis	Pericardium opaque with a purulent exudate. May be complicated by fibrosis leading to constrictive pericarditis.	Cardiomegaly with pericardial effusion *MRI:* May demonstrate thickened pericardium (≥4mm)	Bacteria	By direct or indirect invasion of bacteria (eg. bronchopneumonia)
Septicaemia	Generalized bacterial infection with bacterial replication in the circulation. Gram-negative sepsis may result in endotoxin shock. May be complicated by diffuse intravascular coagulation with petechiae in skin and mucous membranes.	Non-specific post-mortem imaging findings		Different agents depending on community acquired or nosocomial. More information of pathogens involved in nosocomial septicaemia in [21]
Gastro-intestinal infections				More information on etiological agents in [21]

Table 1 (continued)

Condition	Macroscopic appearance	Imaging*	Etiological agents	Remarks and references
Hepatitis	Enlarged and congested liver, with green discoloration if cholestasis, +/- pale areas of hepatocellular necrosis. Chronic viral hepatitis may result in fibrosis and cirrhosis. A cirrhotic liver is firm, with a reduced volume and multinodular appearance on the external and cut surfaces.	Non-specific post-mortem imaging findings *CT:* May show decreased parenchymal attenuation (representing oedema)	Viruses: hepatitis A, B, C, D, E virus, Epstein-Barr virus, Cytomegalovirus	Virus detection via PCR on tissue samples, complementary to viral serology
Liver abscess	Cavity filled with a purulent exudate	*Radiographs:* May show air in the biliary tree or within an abscess, right pleural effusion *CT:* Solid round/oval space occupying lesion with central necrosis/fluid. May contain gas *MRI:* Solid round/oval space occupying lesion with central necrosis/fluid with perilesional edema	Gastro-intestinal bacteria: *Clostridium* spp., *Peptostreptococcus* spp., *S. aureus*, gram-negative bacteria -Parasites: *Entamoeba histolytica*	After/during amoebic colitis or as a complication of bacterial peritonitis
Peritonitis	Peritoneal surface appears opaque and erythematous with an associated purulent exudate. Amount of exudate related to the magnitude and duration of the infectious process.	*CT/MRI:* Thickened peritoneum (smooth, irregular or nodular appearance); free or loculated ascites	Gram-negatives, *Enterococcus* spp., *S. aureus*, alpha- and beta haemolytic streptococci, *Clostridium perfringens*, yeasts	Underlying infectious condition (eg. Acute appendicitis, cholecystitis, diverticulitis, salpingitis) or other abdominal condition (trauma, volvulus, cirrhosis, nephrotic syndrome)
Colitis	Oedematous mucosa, haemorrhagic and ulcerated. *Shigella* usually affects the distal colon; hyperaemic mucosa, oedematous, haemorrhagic with prominence of Peyer patches and associated purulent exudates. *Salmonella* is prevalent in ileum and proximal colon. Mucosa is oedematous, haemorrhagic and associated with linear ulcers. *Clostridium* spp. May cause haemorrhage and necrosis of the colon with risk of perforation. *C. difficile* causes pseudomembranous colitis with a firmly adherent fibrinopurulent mucosal exudate.	*CT/MRI:* Multiple fluid-filled bowel loops with thickened walls, ascites, inflammation/stranding of pericolonic fat	Bacteria: *E. coli*, *S. aureus*, *Salmonella*, *Shigella*, *Campylobacter* spp., *Yersinia* spp., *Clostridium* spp.	Bloody diarrhoea
	Colon mucosa is slightly affected		Viruses: Adenovirus type 40 and 41, Astroviruses, Noroviruses, Enterovirus, Rotavirus	

Table 1 (continued)

Condition	Macroscopic appearance	Imaging*	Etiological agents	Remarks and references
Urogenital Infections				
Pyelonephritis and pyonephrosis	White exudate in pelvis	Non-specific post-mortem imaging findings *CT*: Low attenuation (edema) of renal parenchyma, renal calculus, hydronephrosis, abscess/perinephric collection *MRI*: Affected regions are hypointense on T1 and hyperintense on T2 compared to normal renal tissue	Gram-negatives	
Pelvic inflammatory disease	Yellowish clumps or sulfur granules	*CT/MRI*: Smooth peritoneal thickening, stranding/haziness of pelvic fat, fluid-filled fallopian tubes with thickened walls, tubo-ovarian or pelvic abscesses	*Actinomyces* spp. *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis*	Associated with intra-uterine devices

*HACEK group: *Haemophilus parainfluenzae, Aggregatibacter aphrophilus, Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae*

**Other less frequent pathogens causing myocarditis are: *Epstein-Barr virus*, *Cytomegalovirus*, other respiratory viruses, *Chlamydia psittaci*, *Candida albicans*, *Toxoplasma gondii*, *Trichinella* sp

in patients with advanced HIV infection. Such findings concur with those from conventional invasive autopsies performed in other studies [42].

Swab samples, either nasopharyngeal or oropharyngeal, for SARS-CoV-2 can be taken without damage to the body, before performing the MIA protocol [43]. Swabbing the upper respiratory tract, particularly the nasopharyngeal site, is relevant for post-mortem diagnosis of viral respiratory pathogens [44] while the throat is relevant for measles [45].

Prior to procuring needle tissue samples, the skin should be cleaned with sterile water and disinfected either with alcohol-based solution containing chlorhexidine or iodine. Under the suspicion of legionellosis, it is particularly important to avoid contact with tap water to prevent contamination [41]. Blood samples are collected into an EDTA-containing tube, and an aerobic blood culture bottle. CSF is collected into a sterile tube for analysis and stored at -80°C in Eppendorf Safe-Lock Tubes. Tissue samples obtained by needle biopsy are placed both in thioglycolate broth and in lysis buffer for microbiological analyses. Similarly, samples from these sites and from the heart, spleen, kidney, uterus and skin can be collected for histopathological examination. MIA is ideally performed within 24 hours of death to minimize the effects of PMBT. MIAs performed after 24h after death can still render reliable diagnostic results, although in this situation the contribution of some microorganisms such as *Enterobacteriaceae* and *Pseudomonas* spp. might be overestimated [46] and may reflect bacterial translocation rather than true infections. This translocation may become a larger problem given the delay in performing MIA while waiting for the result of a SARS-CoV-2 PCR taken after admission of a body in the forensic pathology laboratory.

Castillo et al. [32] compared MIA to conventional invasive autopsy. Within 24 hours of death, the authors disinfected and sampled the skin, blood and CSF. This was followed by targeted collection of tissue using needle biopsies for microbiological and histopathological analysis from the liver, lungs, bone marrow and central nervous system [47]. The conventional autopsy was performed immediately after MIA. MIA sensitivity was highest for disseminated infections (98%, 95% Confidence Interval (CI) 87-100%) and lowest for pulmonary infections (79%, 95% CI: 58-93%) due to difficulties in targeting lesions within the lung on blind needle biopsy. MIA specificity was highest for gastrointestinal infections (100%, 95% CI 97-100%) and lowest for disseminated infections (99%, 95% CI 92-100%). The single most frequent infectious COD identified in this study was *M. tuberculosis* complex. [32, 48].

In an innovative study, Van der Linden et al. [49] used needle biopsies under imaging guidance during MIA to

collect samples from the heart, liver and kidney. The authors found that the integrity of the patient's RNA in these samples was larger than in samples subsequently obtained by conventional autopsy, though glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was lower in samples with a PMI greater than 15 hours. Needle biopsies had also been used during MIA with CT-guidance to perform PCR analysis for the presence of bacterioplankton DNA in lungs, spleen, kidney and brain [50] as a diagnostic marker in drowning six days after the discovery of the body. Each samples wascollected using a new sterile cutting needle after cleaning the skin with alcohol and was stored at 4°C in sterile containers without preservative until processing.

Protocol for the collection of microbiology samples at MIA in adults

At present, the literature reflects that there is insufficient worldwide experience to allow a definitive recommendation on how and when samples should be collected for microbiological investigation during MIA. Relatively few studies have addressed this issue, and almost all have been undertaken in areas of high HIV and tuberculosis prevalence [47, 51]. Microbiological investigation during adult MIA is likely to have a lower diagnostic yield with longer PMIs and in areas of low HIV prevalence. It is clear that samples can be successfully obtained for microbiology at MIA with or without imaging-guidance. Provided these have been obtained after first appropriately disinfecting the skin, these samples can be submitted to a wide variety of microbiological investigations, including serology, culture and PCR-based analyses (Table 2). What remains unclear is how many biopsies should be obtained from each site, whether all samples should be collected in every MIA or whether sample collection should be directed by the clinical history and what impact the PMI has on the sensitivity and specificity of such investigations. These are areas for future research.

For several BSL3 pathogens, such as HIV, *M. tuberculosis* complex and SARS-CoV-2, it is difficult to determine the duration of infectiousness of the body. Tuberculosis could be infectious for more than 12 months [52], HIV and SARS-CoV-2 may be detected by PCR for several days post-mortem [53, 54]. To the best of our knowledge the duration

Table 2 Sampling collection during adult MIA for microbiological investigation

Tissue/Body fluid	Site of collection	Means of collection	Type of analysis
Blood	Subclavian vein or heart	Needle aspiration	Direct bacterial culture Virology/serology for viral hepatitis, HIV Molecular analyses*
Cerebrospinal fluid	Occipital approach to the cisterna magna	Needle aspiration	Direct bacterial culture Molecular analyses Antigenic analyses
Urine	Suprapubic aspiration	Needle aspiration	Direct bacterial culture
Pleural effusion	Aspirate via chest wall	Needle aspiration	Direct bacterial culture
Ascites	Aspirate via abdominal wall	Needle aspiration	Direct bacterial culture
Brain	Trans-ethmoidal approach	Cutting needle biopsy	Direct bacterial culture Molecular analyses
Bone marrow	Anterior superior iliac crest	Bone marrow aspiration	Direct bacterial culture
Nasopharyngeal	Nasopharynx	Swabs (Amies/viral transport media/sodium chloride)	Molecular analyses Antigenic analyses
Lungs	Via chest wall	Cutting needle biopsy	Direct bacterial culture Molecular analyses
Spleen	Via left lateral abdominal wall	Cutting needle biopsy	Direct bacterial culture Molecular analyses
Liver	Via right lateral abdominal wall	Cutting needle biopsy	Direct bacterial culture Molecular analyses
Kidney	Via posterior abdominal wall	Cutting needle biopsy	Direct bacterial culture

Samples that can be collected during adult MIA for microbiological investigation. The range of samples collected will depend on the population being served, the clinical history and the resources available. A new sterile needle should be used for each biopsy. [49, 51]

*Molecular analyses either for bacteria or viruses are included in the table in those samples in which they are most frequently useful.

of their infectiousness, i.e. isolation in viral culture in dead human bodies, is not known. Therefore, it is worthwhile taking swabs or punctures for molecular analyses for these BSL3 pathogens, following stringent safety measures [26] even with post-mortem intervals of more than 24 h.

The MIA in stillbirth, neonates and children

Microbiology sampling in pediatric MIA can be obtained using the protocol described above for adult patients (Table 3) [47, 51, 55, 56]. Successful implementation of pediatric MIA into routine clinical practice requires further development and has many issues to consider in order to ensure that the best possible service is provided [22, 23]. However, provided that MIA is undertaken jointly by pediatric pathologists and radiologists, it could be an acceptable alternative to conventional autopsy in selected cases [50]. Currently, the pediatric MIA uses MRI rather than CT [57, 58]. The examination of the placenta becomes an invaluable tool in the post-mortem examination of stillbirth, and in neonatal deaths the study of the placenta becomes key, providing significant information in more than 50% of the MIA cases [59].

In low/medium income countries, where resources are not available, some studies suggest using minimally invasive tissue sampling (MITS) with available clinical data, for attributing underlying and immediate causes of neonatal deaths [60].

In pediatric post-mortems swab samples for SARS-CoV-2 a rectal swab should be added in addition to the nasopharyngeal swab used in adults [61].

MIA in developing countries

Most of the world's population dies without qualified medical personnel either certifying their death or identifying a specific cause. The conventional invasive autopsy is indisputably the gold standard for COD determination. However, it is rarely performed in low-income countries due to the lack of resources, trained available pathologists, poor acceptability and the fact that in these countries most of the deaths occur outside the hospital. Minimally invasive techniques have been proposed as an alternative to the traditional complete autopsy to better refine the mortality statistics in these settings and therefore contribute to effective public health interventions.

In 2013, the Barcelona Institute for Global Health (ISGlobal) started the CaDMIA research project (validation of the MIA tool for COD investigation in developing countries), funded by the Bill & Melinda Gates Foundation, aiming to validate the use of a less invasive methodology for COD ascertainment. The MIA protocol is based on the collection of post-mortem biopsies from key organs (central nervous system, lungs, heart, liver, kidney, bone marrow, spleen and skin) and fluids (CSF and blood). MIA and conventional invasive autopsy samples are studied using traditional and

Table 3 Standard sampling collection during pediatric MIA for microbiological investigation [49, 51, 55, 56]

Tissue/Body fluid	Site of collection	Means of collection	Type of analysis
Nasopharynx/ Throat	Nasopharynx Throat	Swab with transport media (Amies/ viral)	Direct bacterial culture Molecular analyses* Antigenic analyses
Blood	Heart	Needle aspiration Through chest wall or during thoracoscopy (according to procedure)	Bacterial culture Molecular analyses Antigenic analyses
Cerebrospinal fluid	Occipital approach to the cisterna magna or a lumbar puncture	Needle aspiration	Direct bacterial culture Molecular analyses Antigenic analyses
Urine	Suprapubic aspiration	Needle aspiration	Direct bacterial culture
Lungs	Through thoracoscopy	Tissue sample	Direct bacterial culture Molecular analyses
Additional tissues: -Liver, central nervous system) - Bone marrow		Biopsy needles (14G+16G)	Direct bacterial culture Molecular analyses
Bowel content	Through laparoscopy	Incision of bowel, which is sutured thereafter.	Direct bacterial culture Molecular analyses Antigenic analyses
Rectal swab	Rectum	Swab (viral transport media)	PCR SARS-CoV-2

*Molecular analyses either for bacteria or viruses are included in the table in those samples in which they are most frequently useful.

advanced histopathological and microbiological techniques. Pathology and microbiology results are analyzed by a multidisciplinary team in order to reach a consensus regarding the most plausible COD, both for the MIA and the conventional autopsy. The procedure involves the collection of 20mL of blood and CSF and puncture of liver, lungs, heart, spleen, kidneys, bone marrow and brain in all cases plus the uterus in women of childbearing age, using biopsy needles [22, 55, 56]. The type and main characteristics of the different needles used in the MIA procedure for each particular biopsy, and the sites of puncture and the number of samples to be obtained are summarized in Table 4 [47]. The organs are presented in the order in which the samples are collected.

Extensive microbiological investigations are included to identify infectious COD [51]. The sampling and testing scheme for microbiology includes a universal screening for several key pathogens (i.e. HIV, HBV, malaria) and bacterial/fungal culture of biological fluids and tissue samples. In addition to universal screening, frozen aliquots of plasma, CSF or other biological fluids and tissue samples in nucleic acid-preserving lysis buffer are collected for molecular assays (i.e. broad-spectrum PCR assays and specific qPCR tests) guided by histopathological findings. Preliminary results suggest that CSF and lung tissue are among the samples offering a better performance for microbiological analysis to detect an infectious COD. The MIA tool has recently been validated for neonates, children, maternal deaths and other adult deaths from Mozambique [32, 55, 56, 62]. In these validation studies, the concordance between MIA and conventional complete autopsy is moderate for neonates and maternal deaths (kappa = 0.404 and 0.485 respectively) and substantial for children and adults (kappa = 0.704 and 0.732 respectively). The overall concordance between the MIA diagnosis and conventional invasive autopsy was 75.9% (85/112). The concordance was higher for infectious diseases and malignant tumors (63/80 (78.8%) and 13/16 (81.3%), respectively) than for other diseases (9/16; 56.2%). The specific microorganisms causing death were identified in the MIA in 62/74 (83.8%) of the infectious disease deaths with a recognized cause.

Table 4 Type and main characteristics of the different needles used in the MIA procedure for each particular biopsy, sites of puncture and number of samples to be obtained [47]

	Needle	Type	Gauge	Needle length (mm)	Site of Puncture	Volume/Number of samples for microbiology	Number of samples for histology
Cerebrospinal fluid	Quincke Spinal [a]	Manual	20	100	Occipital puncture	20 mL	-
Blood	Quincke Spinal	Manual	20	100	Supra/infra-clavicular or left ventricle	20 mL	-
Liver	Monopty*	Automatic	14-16	115	Anterior right axillar line, 11^{th}-12^{th} intercostal space	2 cylinders	6 core biopsies
Lungs and heart	Monopty*	Automatic	14-16	100	Right and left clavicular region down to the diaphragm for microbiology samples. Multiple random thoracic punctures for pathology	2 from left lung, 2 from right lung	6 core biopsies from each side
Bone Marrow	T-Lok ™ Trephine**	Manual	8	100	Anterior iliac crest	Half of the cylinder	Half of the cylinder
Central nervous system	Monopty*	Automatic	16	200	Occipital puncture Trans ethmoidal puncture. Perforation of the cribriform plate with the bone marrow trephine to reach the cranial cavity	2 cylinders from each approach (occipital and trans-nasal)	6 core biopsies from each approach
Skin	Biopsy punch [aa]	Manual	5 mm	-	Macroscopically detected lesions	-	2 -3 biopsy punches

[a]Becton Dickinson, Franklin Lakes, NJ, USA, [aa]KAI Europe GMBH, Solingen, Germany, *BARD Biopsy Systems, Tempe, AZ, USA, **Mana-Tech Ltd, Staffordshire, UK

Discussion

PMM has shown to be useful to detect an infectious COD, though it must be interpreted with caution, and in the context of the clinical history (including ante-mortem microbiology) as well as imaging, macroscopic and microscopic findings [9, 10]. Where there is concordance between PMM, the history and the autopsy findings, it is reasonable to attribute the identified micro-organism as a causative factor. Where concordance is lacking, it is likely that the microbiological findings are spurious and represent contamination or PMBT [4].

Laboratory interpretative criteria for post-mortem bacterial culturing have been previously defined by many authors [11, 12, 63–68]. The possibility of sample degradation affecting the detection of nucleic acids and therefore compromising the molecular detection of pathogens should also be considered when dealing with post-mortem samples [65]. However, these recommendations are generally addressed to the interpretation of the results obtained in samples taken during a traditional autopsy.

The PMM protocols using MIA offer an alternative to better ascertain infection under specific settings. As mentioned above, some validation studies have already been performed, showing their value [32, 55, 56, 62]. However, additional investigations in a variety of scenarios and the evaluation of how the PMI affects MIA microbiological results compared to those obtained by a traditional autopsy could be of help for a better estimation of its possible utility under different circumstances. Also, in the MIA setting, more interpretation criteria based on prospective studies including not only post-mortem cultures, but also molecular analyses are required for a better comprehension of PMM.

Key Points

1. This study provides specific guidelines on post mortem microbiology (PMM) techniques in the setting of minimally invasive autopsy (MIA)
2. In low and middle -income countries MIA combines clinical history, external examination, minimally invasive tissue sampling (MITS) and microbiology.
3. In developed countries MIA adds to multi slice computed tomography (MSCT) and/or magnetic resonance imaging (MRI).
4. Swabbing is an additional approach to be considered as part of MIA, as it is useful for SARS-CoV-2 and other respiratory viruses.
5. Where there is concordance between PMM, the history, histology and/or imaging findings, it is reasonable to attribute the identified microorganism as a causative factor. Where concordance is lacking, it is likely that the microbiological findings are spurious and represent contamination or post-mortem bacterial translocation.

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