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ORIGINAL ARTICLE

How is post-mortem microbiology appraised by pathologists? Results from a practice survey conducted by ESGFOR

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Abstract Post-mortem microbiology (PMM) is an important tool in forensic pathology, assisting to determine the cause and manner of death. However, there is a lack of standardisation of PMM sampling. In order to get a better insight into the methods used, the available technical options and developmental needs, ESCMID Study Group for Forensic and Postmortem Microbiology (ESGFOR) members designed a survey aimed at pathologists regarding common practices of PMM in clinical and forensic autopsies. Multiple choice questions were developed based on Cumulative Techniques and Procedures in Clinical Microbiology (Cumitech). The questionnaire was sent to pathologists mainly across Europe and Turkey using SurveyMonkey. The survey had 147 respondents. Although all pathologists were aware of the existence of PMM, 39% (19/49) of the participants were not using it. The three main indications for PMM were: (i) clinical suspicion of an infection not confirmed antemortem (83%), (ii) infectious signs at autopsy (83%) and (iii) as part of a standard protocol for foetal/perinatal or paediatric death (67%). Almost 80% of the participants using PMM stated taking 1–10 samples per case. Of the requested examinations, a general bacteriological culture (96%) and a specific polymerase chain reaction (PCR) assay for a particular infectious agent (34%) were most popular. The most frequent samples were: heart blood (66%), peripheral femoral blood (49%), spleen (64%) and lung (56%). Eighty-nine percent of the participants considered PMM a useful resource when investigating the cause of death. Although there are

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some common uses, this survey indicates that there is a need for improvement towards standardising sampling procedures in PMM.

Introduction

Post-mortem microbiology (PMM) has long been a debatable field [1]. However, recent studies have confirmed that PMM is an established tool in forensic pathology, assisting to determine the cause and manner of death [2–5]. This is most relevant in unexpected deaths, as a microbiological invasion may cause or contribute to rapid death with minimal or no histological inflammation [6, 7]. As a new study group within the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), the ESCMID Study Group for Forensic and Postmortem Microbiology (ESGFOR) recently proposed guidelines for PMM sampling according to different clinical scenarios [8]. This proposal was considered a first step in the standardisation of current sampling protocols in use in different European countries. In a subsequent step, pathologists were invited to participate in a survey addressing current practices and gaps in the use of PMM in clinical and forensic autopsies. The aim of the survey was to collect basic information about: current use of PMM by participants across different centres and countries, sampling protocols in use and options available. The survey results were seen as a useful tool to help determine future required developments in the field.

Materials and methods

A series of multiple choice questions was developed by ESGFOR members based on Cumulative Techniques and Procedures in Clinical Microbiology (Cumitech) on PMM [1]. Invitations to fill out the questionnaire were sent mainly across Europe and Turkey by ESGFOR members. They invited both individually more than 300 pathologists and collectively national pathology societies and national forensic associations or pathologists with more than 1000 pathologist members. Participants used a web-based software development which allows real time feedback and data collection (http://www.SurveyMonkey.com).

The questionnaire was launched on September 17th, 2015. The survey was closed on November 20th, 2015. A descriptive analysis of the data was performed. A survey response was considered when at least one of the questions was answered.

The questions are presented in the Supplementary material.

Results

There were 147 responses to at least one question of the survey. Answers were received from 16 countries. The countries with the highest number of participants were Spain ($n = 56$), UK ($n = 19$), Italy ($n = 18$) and Turkey ($n = 17$). Responses were also received from Australia, Belgium, Benin, Finland, France, Germany, Ireland, the Netherlands, Romania, Switzerland, Tunisia and the United Arab Emirates (each of them with five or less participants).

As not all participants responded to all questions, responses were expressed as a percentage of the total received for each question.

Participants reported that there was an average of 271 clinical autopsies performed per year per centre ($n = 47$ respondents), compared with an average of 610 forensic autopsies per year per centre ($n = 87$ respondents). Centres differed in their activities; some performed both clinical and forensic autopsies ($n = 16$), while others focused on either clinical or forensic autopsies. Forensic microbiological analyses were funded by the state in 69% (55/80) of the cases.

Training for performing autopsies was reported by 72% (79/109) of the participants. This ranged from an in-house training to a postgraduate course or specialised master training.

In addition, of the 49 (33%) participants not sampling for PMM, more than half (25/49, 51%) were aware of this ancillary investigation and had requested it previously; 35% (17/49) were interested in PMM and 39% (19/49) stated that this was not currently available at their place of work/country.

As shown in Table 1, a high percentage of pathologists (72/83, 87%) made use of PMM. Remarkably, forensic pathologists seemed to make more use of PMM than clinical pathologists. Respondents from all participating countries except Benin and the United Arab Emirates stated using PMM.

Table 1 further shows the general data regarding the autopsies performed in different centres.

Analyses requested

The three main indications for performing PMM were: (i) clinical suspicion of an infection not confirmed antemortem (83%), (ii) the identification of signs of a possible infectious disease during autopsy (83%) and (iii) as part of a standard protocol for foetal/perinatal or paediatric death (67%). Interestingly, medical negligence was an indication for 24% of the respondents. In rare instances, specific indications are mentioned, such as maternal deaths, death of transplant patients or patients having died after more than 72 h of hospitalisation at intensive care units.

The most popular requested analyses were a general bacteriological culture (96%) and a specific polymerase chain reaction (PCR) assay for a particular infectious agent

Table 1 General data regarding the autopsies performed in different centres

Question		Answer	No. of answers
Covered population (no. inhabitants)		Mean $2*10^6$ (range: $90–25*10^6$)	78
No. of clinical autopsies/year per centre		Mean 271 (range: 0–4500)	47
No. of forensic autopsies/year per centre		Mean 610 (range 0–5000)	87
PMM analysed for other centres? Yes		48 (34%)	141
Who performs/assists during autopsy?			142
	Forensic pathologist	93 (65%)	
	Technician	92 (65%)	
	Clinical pathologist	49 (35%)	
	Other	26 (18%)	
Rate of personnel trained for autopsy		72%	109
Do you request/perform PMM? Yes		83 (86%)	97
Indications for PMM			87
	Standard protocol (childhood death)	58 (67%)	
	Suspected death, any age	32 (37%)	
	Confirmation of antemortem suspicion of infection	72 (83%)	
	Suspected lesions during autopsy	72 (83%)	
	Medical negligence	21 (24%)	
	Other	9 (10%)	

PMM post-mortem microbiology

(34%). Protozoal diagnostics (6%) and 16S rRNA sequencing (6%) were the least requested analyses (see Fig. 1). While the numbers were small, clinical pathologists requested more often fungal and mycobacterial cultures, viral culture or serology and viral molecular analysis than forensic pathologists.

Of those participants who did not routinely request PMM analysis, 22/59 (37%) used it sporadically for a specific case. Forty-nine percent (39/80) of the respondents asked for a more detailed analysis according to the first microbiological results.

Health and safety

General questions were included in the survey in order to enquire about the protection of the autopsy personnel. Limitation of the number of personnel to minimise contamination in the autopsy room was reported by 42% (34/81) of the participants. The safety measures most frequently reported were: personal protective equipment (74/83, 89%), special suits for high-risk autopsies (38/83, 46%) and laminar airflow (33/83, 40%). Sterile gloves for sampling were only used by 55/83 (66%) of the participants.

PMM sampling

A formal protocol for PMM sampling was followed by 53/84 (63%) of the respondents. The types of samples taken to investigate a possible sepsis are shown in Fig. 2. Only 62% (49/79) of the participants disinfected the target place prior to sampling. Eighty-three percent of the respondents (66/80) stated that they took from 1 to 10 samples per case. Samples

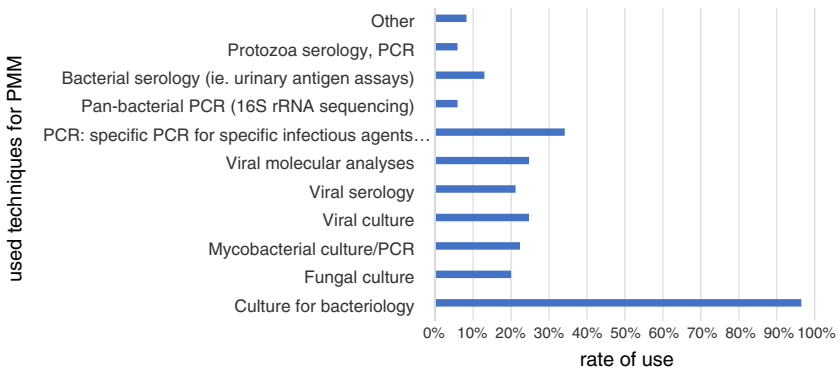

Fig. 1 Type of microbiological analysis requested by pathologists

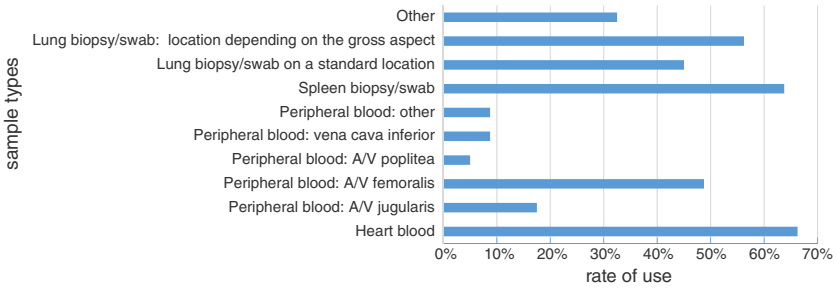

Fig. 2 Sample types taken for sepsis diagnostics

were most frequently taken before the manipulation of solid organs and intestine (72/80, 90%), and using sterile bistouries or flamed knives for each specimen (62/80, 76%). Blood was sampled in many different ways and from different places in the body. Containers to store blood included: gel separator tubes (9/79, 11%), citrate blood tubes (13/79, 16%), EDTA tubes (29/79, 37%) and blood culture bottles (41/79, 52%). Fifty-one percent (39/76) of the respondents reported taking lumbar fluid percutaneously via cisterna magna. Organs were most often sampled either after searing of the organ and taking a tissue sample (24/80, 30%) or swabbing the disinfected and incised organ/area (25/80, 30%). Tissue samples were mostly stored in a sterile jar without additives (49/71, 71%). All types of swabs were used, with a slight preference for swabs with viral transport medium (36/80, 45%), swabs in Amies transport medium (30/80, 38%) and dry swabs (28/80, 35%).

Transport of samples was performed following the chain of custody criteria in 75% (59/79) of cases. More than half of the participants (47/81, 58%) understood that samples should be sent to the laboratory within 24 h after the autopsy and that they should be stored in a refrigerator (56/78, 72%) up to transport. Forty-four percent (35/79) of the participants made use of a specialised laboratory for microbiological analysis. The sample types taken the most (>50%) were heart blood (53/80, 66%), peripheral femoral blood (39/80, 49%), spleen (51/80, 64%) and lung biopsies according to gross aspect (45/80, 56%).

Microbiological analyses and their usefulness

Many of the participants (54/73) did not know how samples were inoculated in the microbiology laboratory. Among those who answered affirmatively, one can distinguish different techniques of inoculation: plain inoculation into broth ($n = 6$), striking tissue on solid agar ($n = 3$) or homogenisation of tissue and placing this homogenate into broth ($n = 10$). The incubation interval of cultures reported ranged between 2 and 7 days. A high number of respondents (55/76, 72%) knew that the laboratory performed molecular analyses.

Most of the respondents (68/76, 89%) considered that PMM was a useful resource when investigating the cause of death. Among the circumstances believed to decrease the accuracy of PMM, the pathologists mentioned: a long post-mortem interval (32/79), too many contaminating microorganisms (21/79), antemortem antibiotics (11/79), immunocompromised patients (6/79) and putrefaction (1/79). Seventy-four percent (53/74) of the respondents declared that, in their institution, culture results were interpreted by the microbiologist. The main way of interaction with microbiologists was via phone or email (53/78, 69%) or at multidisciplinary meetings (12/78, 16%).

Discussion

One hundred and forty-seven clinical or forensic pathologists participated in the ESGFOR survey addressing current use and gaps in PMM. A major limitation to the survey was that, because many respondents did not answer all questions, the denominator data were different for each question. Only 84 (84/147, 57%) participating pathologists filled out the survey completely.

In a large series of more than 500 ‘sudden unexpected death in infancy’ autopsies, Weber et al. [9] showed that, in the “explained death group,” the most important components of the autopsy determining the cause of death were: macroscopic examination (30%), histological examination (46%) and microbiological investigations (19%). A recent study by Morentin et al. [10] analysed 56 cases of sudden death of infectious origin in children and young adults. The authors described that the organism responsible for the infection was detected in 17 of the 23 autopsies in which microbiological analyses had been performed [10]. This survey showed that 89% (68/76) of the participants considered PMM a useful resource when investigating the cause of death. Sixty-three percent of the respondents reported to follow written guidelines or protocols: reference is made to local procedures, national recommendations and published literature. This knowledge is reflected in the use of heart blood, followed by a spleen biopsy as the preferred sample to exclude or confirm an undiagnosed sepsis [8].

From the lab tests requested, it was clear that protozoal diagnostics and newer tools like 16S rRNA sequencing were not well known. Only 20–25% of the respondents indicated the use of virology, mycobacterial or fungal cultures. However, it was stimulating to learn that specific PCR for

targeted pathogens was the second most frequent analysis requested after the bacteriological culture (Fig. 1). Enhancing a direct communication with pathologists is mandatory in order to make them aware of new available technologies in use at the microbiology lab.

Although there are some common practices and some centres use local microbiology sampling protocols, this survey showed that there is significant room for improvement towards standardising sampling procedures in PMM. For example, the introduction of a protocol for microbiological sampling for specific clinical circumstances of death will allow a more precise investigation of each case, as well as provide uniformity across participating countries. Also, techniques such as next generation sequencing could be of help for samples containing a mixture of microorganisms and where 16S rRNA sequencing falls short. Combining routine culture techniques and real-time PCR targeting specific genes for well-known pathogens with next generation sequencing may be important in the understanding of findings in post-mortem samples and, eventually, in the interpretation of results regarding possible infectious causes of death. These novel techniques may be of value in future scientific studies to detect pathogens when traditional techniques have yielded negative results and the cause of death is an infection. They can also be useful to explore the commensal and pathogenic flora of several human body systems in sudden death cases. Also, post-mortem time can be evaluated based on the change in the composition of particular microbiomes in the human body. These types of studies might help in clarifying the interpretation of PMM given the many contaminating microorganisms found in these samples.

Another limitation of this study is that we are not aware of the exact number of invited respondents in the survey. The questionnaire was sent by ESGFOR members to at least 1300 pathologists by means of mails to national pathologists societies and fora, as well as to individual specialists. When the survey was sent individually, the response rate was about 30%, whereas when it was sent collectively, the response rate was lower (about 5%).

A last limitation in this study was the fact that answers from either clinical or forensic pathologists could not be easily distinguished since some respondents performed both tasks and answered consequently. Consequently, it was difficult to see differences in the use of PMM between both groups of pathologists.

As a conclusion, the development of a common space to share practical experience, educational activities and promote research aimed to improve the post-mortem diagnosis of infectious diseases is of paramount importance for microbiologists and pathologists. Thus, collaboration between scientific societies of these two disciplines is strongly recommended. As a first step, an agreement between the ESCMID and the European Society of Pathology (ESP) has already been signed and, therefore, common activities are forthcoming.

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Compliance with ethical standards

Funding There was no funding received for this survey or for the writing of the manuscript.

Conflict of interest The authors have no conflict of interest to report.

Ethical approval Ethical approval was not required for this survey, no patient data have been used. Accordingly, no informed consent forms were used.

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