MINIMALLY INVASIVE AUTOPSY - AN ADVANCED TECHNIQUE IN DETERMINING THE CAUSE OF DEATH

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Summary

Complete diagnostic autopsy (CDA) is the gold standard in determining the cause of death. However, according to the statistics of the World Health Organization, the practice of CDA has been on a dramatic decline in high - income countries or has not been recorded consistently in low - and middle - income countries, including Vietnam, for the last few decades. This is due to capacity and resource issues as well as cultural and religious factors regarding management of corpses. As a result, the development of quick, less invasive procedures becomes necessary in order to improve the statistics of cause of death worldwide. One such procedure that has the potential to serve as an alternative to complete diagnostic autopsy is minimally invasive autopsy (MIA). This procedure involves using hollow needles to take samples from different tissues and fluids from key organs before combining histology and microbiology to determine the cause of death. This paper aims to explore this novel procedure as well as its potential in becoming the dominant post - mortem examination, especially in the context of infectious causes of death.

Key words: minimally invasive autopsy, minimally invasive tissue sampling, needle autopsy, postmortem examination.

INTRODUCTION

Complete diagnostic autopsy (CDA) is the gold standard in determining the cause and the manner of death of a patient. The procedure for autopsy is essential in medical education for students and physicians, public health monitoring, and identifying new and changing diseases[1]. However, according to the statistics of the World Health Organization (WHO), the rate of CDA has been in a dramatic decline in high-income countries for the last few decades[2]. The statistics in low- and middle-income, including Vietnam, are even worse, as there is no national program collecting data about the autopsy and the cause of death (CoD). This is due to capacity and resource issues as well as cultural and religious factors regarding the management of corpses. Therefore, the development of quick, less-invasive post-mortem examination methods is necessary to improve the statistics of autopsy as well as to monitor diseases. One such procedure is the minimally invasive autopsy (MIA) or minimally invasive tissue sampling (MITS). MIA is a protocolized hollow needle-based post-mortem examination, designed as an acceptable proxy of the gold standard of CDA. MIA involves inserting fine needles into the body and collecting small amounts of tissue and body fluids from key organs like the brain, lungs, spleen, heart, bone marrow, and uterus in women of childbearing ages[3]. These samples are then analyzed through histopathological and microbiological techniques to provide useful information about the CoD. This paper aims to explore the procedure of this novel procedure in detail as well as its potential in becoming the dominant post-mortem examination, especially in the context of infectious diseases.

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THE STANDARD PROCEDURE FOR MINIMALLY INVASIVE AUTOPSY

Before the development of a standard of procedure (SOP) for MIA, most studies considered the use of Tru-Cut needles (Travenol Laboratories, Deerfield, Illinois, USA), consisting of an inner solid needle, the obturator, and an outer hollow needle, combined with standard histology as the main way to collect useful information about the CoD of the patients[4;5]. The procedure of modern MIA remains mostly the same, with the exception of the help from advanced technology like magnetic resonance imaging (MRI), computed tomography (CT) scans, and portable ultrasonography for increased precision as well as from specialized needles for a higher rate of success. Among different SOP from multiple organizations and networks worldwide, the SOP of the CaDMIA project, developed by the University of Barcelona and later tested/refined at the Maputo Central Hospital and the Centro de Investigação em Saúde de Manhiça in Mozambique, has been validated in multiple studies in low- and middle-income countries[3;6;7;9;10]. The procedure starts by evaluating abdominal organs like the liver, kidneys, spleen with an ultrasound (US) scan device to look for lesions or abnormal fluids (ascites, pleural effusions) and to record the locations of organs. In women of childbearing age, a scan of the pelvis is also performed. Once the scanning is finished, the ultrasound gel is removed by cellulose paper and the skin areas to be punctured are cleaned with tap water and then disinfected with 96° alcohol and iodine for 5 minutes each. The sampling process initiates with the collection of cerebrospinal fluid (CSF) by occipital puncture and blood by puncture of supraclavicular or infraclavicular veins. Up to 20 mL of each fluid is collected, even fluids from ascites and pleural effusions that are discovered during the US scan. After the bodily fluids have been collected, specimens of major organs are taken, including the liver, the lung, the heart, the kidneys and the central nervous system (CNS). The technicians are required to take 6 biopsies at each organ, using the same entry point. The sampling should target different regions within the organ to obtain an adequate representation of the whole organ. In specific cases, samples from the bone marrow, the spleen, and the uterus are collected to study.

In addition to progressive radiology and instruments, compared with the primitive MIA, modern MIA also requires microbiological investigation besides histology. For histological analysis, all samples are fixed in 10% neutral buffered formalin for 4 hours, passed into distilled water, embedded in paraffin before being stained with hematoxylin and eosin according to the standards of histology. When necessary, ancillary histochemical and/or immunohistochemical are used to confirm the diagnosis. For the microbiological analyses, universal screening for human immunodeficiency virus (HIV), hepatitis B/C virus (HBV/HCV), *Plasmodium falciparum* as well as the bacterial/fungal culture are performed. *Plasmodium falciparum* is detected via Polymerase Chain Reaction (PCR) while the presence of antibodies against HIV-1/2 and HBV/HCV surface antigens are tested from plasma centrifuged from the collected blood. With HIV-positive cases, the viral load is determined and additional tests are performed; in particular, real-time PCR in CNS and CSF samples for *Toxoplasma gondii, Mycobacterium tuberculosis,* and *Cryptococcus spp.*; real-time PCR in lung samples for *Pneumocystis jirovecii, Cryptococcus spp., Mycobacterium tuberculosis*, and *Talaromyces marneffei.* The remaining blood volume is cultured for the detection of bacterial or fungal growth. Positive blood cultures are subjected to Gram stain and sub-cultured in appropriate agar plate[3;7].

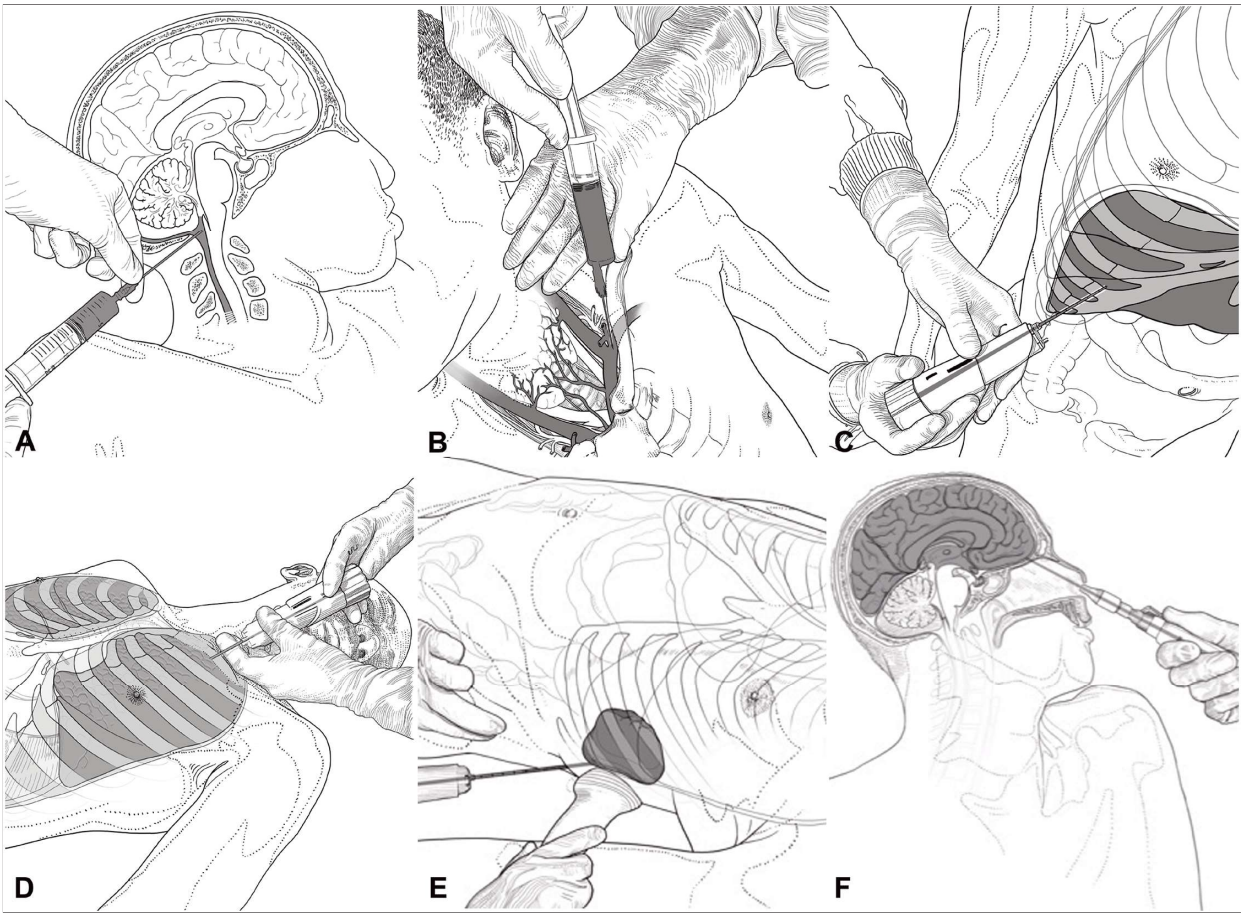

Figure 1. Procedures for the collection of CSF (A), blood (B), liver (C), lung (D), spleen (E), and CNS (F) (designed by Xabier Sagasta)[3]

Table 1. Types of different needles in MIA procedure for each particular biopsy, puncture sites and number of samples to be obtained[3]

Tissue	Needle	Type	Gauge	Needle length (mm)	Puncture site	Microbiology samples	Histology samples
CSF	Quincke Spinal	Manual	20	100	Occipital puncture	20 mL	-
Blood	Quincke Spinal	Manual	20	100	Supra/Infra - clavicular or left ventricle	20 mL	-
Liver	Unicut	Manual	14	115	Anterior right axillar line, 11th - 12th intercostal space	2 cylinders	4 - 6 cylinders
Lungs	Monotopy	Automatic	14	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	4 - 6 cylinders from each side
Heart	Monotopy	Automatic	14	100	Left thoracic region 5th intercostal space in a parasternal point	-	2 cylinders
Spleen	Monotopy	Automatic	14	160	Posterior left axillar line in the 11th - 12th intercostal space (locate with US scan)	-	2 cylinders
Kidneys	Monotopy	Automatic	14	160	Upper abdominal/lumbar area (locate with US scan)	-	2 cylinders

Tissue	Needle	Type	Gauge	Needle length (mm)	Puncture site	Microbiology samples	Histology samples
Bone Marrow	T-LokTrephine	Manual	8	100	Anterior iliac crest	0.5 cylinder	0.5 cylinder
CNS	Biomol	Semi Automatic	16	200	Trans-ethmoidal puncture. Perforation of the cribriform plate with the bone marrow trephine to reach the cranial cavity	2 cylinders	4 - 8 cylinders
Uterus	Monopty	Automatic	14	160	Central suprapubic region (locate with US scan)	2 cylinders	4 - 6 cylinders
Skin	Biopsy punch	Manual	-	5	Macroscopically detected lesions	-	2 - 3 biopsy punches

MINIMALLY INVASIVE AUTOPSY VALIDATION COMPARED WITH COMPLETE DIAGNOSTIC AUTOPSY

In the early studies comparing MIA with CDA, the concordance between the two methods used to be low, leaving the authors to conclude that CDA was preferable to MIA. A large - scale study that compares primitive MIA with CDA was published in 1957 in North America[4]. The investigators found that among the 50 cases undergoing both MIA by Vim - Silverman needles and CDA, where the pathologists conducting MIA were blinded to the clinical diagnosis and CDA results, there was a discrepancy of 52% (26 cases) between the 2 techniques. The most common causes of discrepancies were myocardial infarction, pulmonary infarction, cerebrovascular accidents, heart failure, and gastrointestinal hemorrhage, which is similar to many other studies in the period. The next major article comparing MIA and CDA was not published until 30 years later, in 1995 by Foruodi et al. using Tru - Cut needles[5]. Among 21 non - traumatic, non - suspicious cases that underwent MIA and CDA, the CoD was ascertained in 9 cases (43%), compared to the 95% of CDA. It is important to note that the low concordance in these studies might be because the rate of success in obtaining specimens from key organs like heart and kidneys is low; specimens are not representative of the entire organs or system; no advanced technology in providing images for tissue collection and anatomical information is used.

In fact, the rate of concordance has improved over time as more radiology imaging technology and the invention of specialized needles were incorporated into the SOP. A recent study performed by Castillo et al. in 2015 as a part of the CaDMIA project is an example[3]. The standard of procedure in this study introduces the use of US scans before the sampling to precisely locate lesions and organs as well as the combination of different needles for biopsies to increase the chance of success in collecting specimens. As a result, the efficiency of sampling procedure ranges from 66.7% for kidneys to 100% for liver, lung, and CNS while a putative CoD was identified in 83% of the MIA. Follow - up validating studies in the same project confirm that MIA and CDA concordance rate is 75.9% (85/112) for adults, 89% (48/54) for pediatric deaths, 68% (39/57) for maternal deaths, and 83% (15/18) for neonates and stillborns[6,8,9,10]. Although these numbers suggest that MIA is slightly inferior to the gold standard of CDA, MIA is regarded as a valuable and robust alternative because of the potential to identify diseases without physical symptoms through histology and microbiology, the ability to easily achieve consents from relatives, and no prerequisite for doctors to perform the procedure.

MINIMALLY INVASIVE AUTOPSY AND INFECTIOUS DISEASES

As mentioned in the previous section, the MIA procedure includes microbiological investigation to detect etiological agents of infectious deaths. By combining classical microbiological - culture - based techniques and modern molecular microbiology methods, most of which are based on PCR assays, MIA allows the detection of etiological agents in most cases and sometimes even rare agents that CDA cannot identify. In an extensive microbiological investigation via MIA performed by Martínez et al. as a part of the CaDMIA project[7], the detection of at least one etiological agent was detected in the great majority (89%)

of infectious deaths and up to 11 different pathogens were identified, among which *Rhizopus oryzae* and *Acinetobacter baumannii* are rarely reported at the location of the study, Sub - Saharan Africa. Other validating studies of the same project[6,8,9,10] show similar results, with high concordance rate between MIA and CDA for infectious diseases among different CoDs and the identification of etiology in MIA only. In addition, because MIA does not require direct contact with blood and other bodily fluid, the MIA likely involves less risk for the health personnel than CDA, which is of critical importance in the context of infectious diseases.

CONCLUSION

Although the concept of MIA and tissue - based post - mortem examinations have been around for a long time, there is an increase in the number of MIA studies in the last decade. With the advance of technology, especially in radiology, the concordance between the gold standard of CDA and MIA as well as the success rate in obtaining samples is now so significant that MIA is being recommended over CDA as the main way of post - mortem examination.

Table 2. Number of etiological agents by diseases identified by CDA (gold standard), by MIA, and the concordance between CDA and MIA in neonates, children, adults, and mothers

Disease Category	Castillo et al* [6]	Menendez et al* [8]	Bassat et al* [9]	Castillo et al* [10]
Disseminated Infections	41/-/35	-	17/17/8	9/9/19
Pulmonary Infections	24/-/17	-	13/20/11	2/1/1
CNS Infections	13/-/11	-	8/5/3	5/5/5
Septic Abortion	-	-	-	4/6/4
Puerperal Sepsis	-	-	-	2/2/1
Neonatal Sepsis	-	21/21/14	-	-
TORCH Syndrome	-	3/2/2	-	-
Other	2/-/0	3/5/1	4/0/0	-

**The number of etiological agents identified in CDA/in MIA/in both CDA & MIA*

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