Review Article

Collection and Transport of Specimens for Toxicological Analysis in Fatal Aircraft Accident Autopsies - Need for Change

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Abstract

During fatal aircraft accident investigations, post-mortem samples are collected for histopathology and toxicological analysis. A full-fledged toxicology lab is being set up at IAM. This lab will have sophisticated equipment like Gas Chromatography Mass Spectrometer (GCMS), Liquid chromatography Mass Spectrometer (LCMS), and High Performance Thin Layer Chromatography (HPTLC) capable of performing an extensive drug screen. The present policy of sample collection from aviation accident autopsies in Armed Forces has to be upgraded to suit the present times and new equipment. Also the transport of samples from place of accident to IAM needs to be improved. This paper will discuss all relevant issues for collection and transport of aviation accident autopsy specimens.

IJASM 2012; 56(2): 32-38

Keywords: Post-mortem, Toxicological analysis, Specimen Transport, Logistics

Introduction

The flying process involves the interaction between man and the machine, and it is not only the equipment failure, but also the performance impairment and/or inadvertent actions of aviators that might contribute to an fatalaccident . The performance/behaviour-related changes in aviators may be because of the presence of foreign substances in their system. Therefore, globally toxicological analyses and biological sampling from deceased aviators havebeen analysed for the presence of foreign substancesviz.combustion gases, ethanol/volatiles, and other drugs - to establish whether these substance(s) did induce performance impairment/behavioural abnormality and contributed to causation of a particular aviation accident [1,2,3&4]. The presence of drugs would also be suggestive of an underlying pathology for which the individual might have been on medication. This screening process, world over, is now recognized as the Human Performance Associated Post-Mortem Forensic Toxicology. In present day, it is an inherent part of almost every aircraft accident investigation [5].

Following a fatal aircraft accident, post-mortem samples are collected from deceased and toxicologically evaluated in a forensic toxicology facility. In majority of situations, the samples are from pilots and co-pilots. Samples from passengers and other crewmembers are also sometimes evaluated, depending upon the nature of an accident (e.g., an accident involving fire).

Commensurate with establishments in other air forces world over, a sophisticated modern toxicology laboratory capable of a complete drug is being established at IAM Bangalore. The methods employed for autopsy sample collection presently will be discussed along with the methods used . The various proposed changes will then be discussed which can be implemented in our setup.

International scenario

For the investigation of civil aircraft accidents occurring within the jurisdiction of the United

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Table 1: Types, Amounts, and Analytical Suitability of Specimens required by CAMI For Toxicological Evaluation

Specimen Type	Optimal Amount	Analysis Type Suitability	Transport temp
Blood	40 mL	Green-top tube blood for carboxyhemoglobin (COHb), blood cyanide (CN^-) Hemoglobin A1c (HbA1c), and drug analyses Gray-top tube blood for ethanol and drug analyses	2-8^{O}C
Urine	100 mL	Ethanol, drug, and glucose analyses	2-8^{O}C
Vitreous Humor	2 mL	Ethanol and glucose analyses	2-8^{O}C
Spinal Fluid	Available Amount	Ethanol and, perhaps, drug analyses	2-8^{O}C
Gastric Contents	100gm	Need-based drug analysis	2-8^{O}C
Liver	500gm	Ethanol and drug analyses	Frozen
Muscle	300gm	Ethanol and drug analyses	Frozen
Spleen	150gm	Ethanol and drug analyses	Frozen
Lung	100gm	Ethanol and drug analyses	Frozen
Kidney	100gm	Ethanol and drug analyses	Frozen
Brain	100gm	Ethanol and drug analyses	Frozen
Heart	50gm	Ethanol and drug analyses	Frozen

States, autopsied biological samples collected from the victims of ill-fated civil aircraft (air carrier and general aviation) are submitted to the Federal Aviation Administration's (FAA) Civil Aerospace Medical Institute (CAMI) for toxicological evaluation [6]. Such sample submission is coordinated through the FAA's Office of Accident Investigation by the National Transportation Safety Board (NTSB), which is responsible for investigating all US civil aircraft accidents. In the majority of cases, the post-mortem samples are from the aircrew. However, depending upon the nature of an accident— for example, an accident involving fire- samples from passengers and other crewmembers are sometimes collected. These biological samples are routinely analysed for the presence of combustion gases, alcohol/ volatiles, and other drugs. The post-mortem forensic samples are submitted to CAMI in containers. These containers with specimens are shipped to CAMI by an air carrier service for next-day delivery. Details of the samples collected and dispatched to CAMI are given in table-1.

Indian scenario

Department of Aviation Pathology & Toxicology, Institute of Aerospace Medicine, Bangalore is the nodal centre for Pathological and Toxicological analyses of Post-Mortem samples following an Aircraft accident investigation, both civil and military. Currently the procedures are for collection and preservation of autopsy samples are enumerated in table-2.

As per the existing policy the samples are to be dispatched in the frozen state on ice-salt mixture in a thermocol container or thermos flask, the ice in the thermocol container or thermos flask to be frequently replenished by the escort.Practically, all the samples received in the department are being sent in thermos flask/casseroles/ice box with ice. They are being sent by train in 60% cases and in other cases the concerned Medical Officers liaises

Table 2. Procedure for collection & Preservation of autopsy samples as per IAP 4305

Study	Sample	Anticoagulant	Storage and dispatch
Lactic Acid	Blood (5 ml)	Na F (10mg/ml)	Frozen
	Brain	-	Frozen
Carbon monoxide	Muscle	-	Frozen
	Blood	Sterile bottle without anticoagulant	Frozen
Ethanol	Blood	NaF	Frozen
	Brain	-	Frozen

with the authorities and sends them by an IAFcourier. The samples sometimes are kept in the fridge for even up to one week for want of an available means of transport / despatch. The average time taken by the samples to reach IAM has been 5.5 days (Average time calculated for the autopsy samples received at IAM from 2010-2012).

Proposed Procedure

The existing procedure for collection and transport of samples needsa review. The present practiceat times, in past has resulted in receipt of unusable blood/tissue samples at IAM. The suggested changes in sample collection and preservation are presented in table-3.

Discussion

The problems faced and solutions suggested for Preservatives Used for Toxicology and Biochemistry Samples are discussed as below:-

CO Estimation

Presently, blood samples for CO estimation are to be collected in a sterile bottle without preservatives. The CO estimation procedure followed by IAM is as per Van Kampen EJ et al, 1983 [8] and requires whole blood, ie blood collected with a preservative. Both EDTA and NaF[8, 9] are

Table 3: Quantity, Preservative and Temperature for Storage and Dispatch of Autopsy Samples for Toxicological studies

Study	Sample	Quantity	Anticoagulant/ Preservative	Storage &Dispatch
Carbon monoxide	Blood	5ml	Na F (10mg/ml) /EDTA	2-8^{0}C
	Muscle	150-200gm		Frozen
Ethanol	Blood	5ml	Na F (10mg/ml)	2-8^{0}C
	Urine	5ml	Few drops of H_2SO_4	2-8^{0}C
	Vitreous Humour	2-3ml	Nil	2-8^{0}C
Drug assay	Blood	25ml	Na F (10mg/ml)	2-8^{0}C
	Urine	5ml	Few drops of H_2SO_4	2-8^{0}C
Lactic acid estimation (In case where hypoxia is suspected)	Brain	150-200gm		Frozen
	Plasma#	2-5ml	Na F (10mg/ml)	2-8^{0}C
Blood glucose (in case where hypoglycemia is suspected)	Blood	5ml	Na F (10mg/ml)	2-8^{0}C
	Vitreous Humour	2-3ml	Nil	2-8^{0}C

appropriate. It is reiterated that samples collected without preservatives, having only serum and clotted RBCs, are unusable for CO estimation.

It is recommended that NaF (10mg/dl) be used as a preservative for CO estimation. This has an added advantage that any sample in excess can be used for drug assays as and when facilities are created at IAM.

Lactic acid

Blood samples for lactic acid are to be collected in NaF and sent. However, samples sent in this manner will cause the cellular elements in contact with plasma to utilize glucose anaerobically and wouldlead to falsely elevated values [10].

The samples collected in NaF have to be centrifuged at 3000 rpm for 10 min and supernatant plasma separated and sent at 2-8°C. This additional step is suggested as it is only in separated plasma that lactic acid is stable [10]. This separation can be done using a routine laboratory centrifuge present in SMC CSR.

Vitreous Humour

Presently, Vitreous humour is not a part of samples collected for toxicology.

Vitreous humour, has not been a part of the post-mortem samples earlier. It has been proposed to be added to the list of specimens to be sent, so as to assess its utility in aircraft accident autopsies. In case of difficulty in aspirating blood from the body such as delay in body recovery resulting in coagulation/thrombosis of the peripheral blood, vitreous humor is found to be relatively well protected from PM degradation and can be used as a more suitable alternative. Vitreous humour samples are to be sent for estimation of alcohol and glucose [11-13].

Storage and Transport of Samples

All blood samples for CO, Lactic acid and Ethanol after collection in appropriate preservatives are frozen. Freezing and thawing causes lysis of RBCs and results in haemolysed samples. This has been consistently noted in the samples sent to IAM in the past. Anecdotal evidence suggests haemolysis in approximately 50% of the samples. Haemolysis interferes with biochemical and toxicological analysis. These samples need to be transported at 2-8°C. Only brain and muscle tissues need to be frozen. This is same as guidelines available in the FAA document titled "Procedure for collecting and shipping of toxicology specimens" [14].

Transport of Samples

(a) The present policy being followed for transporting the toxicology samples stated that "the samples are to be dispatched in the frozen state on ice-salt mixture in a thermocol container or thermos flask, the ice in the thermocol container or thermos flask to be frequently replenished by the escort. Also the specimen should never be sent by post/SDS"

(b) The existing practices are not adequate as the policy of escort replenishing the ice is impractical and desired temperatures are not maintained and sub-zero temperatures are never attained.

(c) To circumvent this problem, in some cases, samples are sent by scheduled Airforce transport aircrafts. More than delay in transport, the cold chain during transport by service aircrafts is not maintained as dry ice/ gel packs is usually unavailable in remote areas. Moreover during routine halts of the aircraft or unscheduled delays, there is no facility for en route replenishment of dry ice or frozen gel packs. In many instances, water from melted ice has leaked into the samples spoiling them.

Table 4: Comparison of common couriers with temperature controlled logistics in India

	Blue Dart	World couriers	PDP couriers	TNTCouriers
TCL centers	78 (Guwahati is TCL location)	7	4	21
Additional pick up points (agent locations	20 Tezpur and Diblugad are additional pick up points in north east	20 Do not have any centre beyond Guwahati in the north east	27 Do not have any centre beyond Guwahati in the north east	40 Do not have any centre beyond Guwahati in the north east
Temperature loggers with boxes	Present	Present	Present	Present

(d) Hence, the present policy of transport needs to be reviewed so that not only the samples are transported without delay but also proper temperatures are maintained during transport.

It is recommended that samples of such nature should be collected and transported in validated vaccine carriers and cool boxes which contain frozen gel/ice packs to maintain the temp between 2-8 °C for specified periods and dry ice for sub-zero temperatures. A period of 48 to 72hrs after collection is an adequate time frame for the samples to reach IAM without deterioration, provided the cold chain is maintained during storage and during transport. As has been brought out above, there are three types of samples to be transported:-

(a) Brain and muscle - At frozen or sub-zero temperature

(b) Blood, urine, vitreous humour - at 2-8 °C

(c) Histopathology samples - at room temperature

The following alternatives are discussed to transport the samples in time and maintain the cold chain:-

(a) The samples can be sent by service aircraft with escort. This at times may require a special aircraft to be arranged at short notice for sending the samples. In addition the unit will have to make arrangements for dry ice and gel packs, which may replacement enroute. Availability of dry ice in remote locations may have to be ascertained.

(b) The second option available is transport by civil couriers having the facility Temperature controlled logistics (TCL), with validated boxes, temperature loggers and cold rooms enroute. This facility has additional advantage of monitoring the temperatures during transport and replenishing of dry ice and gel packs in case of delay. Both these facilities will not be available in case samples are sent by service aircraft. World over, including Federal Aviation Administration, USA, toxicology samples are sent by couriers such as FedEx having similar TCL (Temperature Controlled Logistics) system.

Both options are acceptable as long as the required temperatures (2-8°C and sub-zero temperatures) are maintained during transport and samples reach IAM within an acceptable period of 48-72hrs after autopsy.

A comparative listofsome of the couriers providing Temperature Controlled Logistics (TCL) in India ispresented in table-4.

Presently, there are many couriers with TCL services having a wide networking in India especially in the North East. They have numerous TCLcentres with additional pick up points where they send their cold boxes by air in 24 hours from the Metros. Their TCL centres have cold rooms and facilities for replenishing dry ice/ice packs. They also have centralized TCL coordinators who can be contacted at the time of crash.

Cost of transporting a sample by courier with TCL services from Tezpur (which is a non TLC centre) to Bangalore is approx.‘ 49,000/- .The cost of transporting the specimens from Tezpurto Bangalore (Distance 3150 km, 4 hours flight time) would be about (‘2.43 lac /hour) ‘ 9.72 lacs.

Solution Suggested

In view of the above the following is recommended:-

(a) In eventuality of fatalities during aircraft accident, availability of service aircraft for transport of specimen should be ascertained. If unavailable, then couriers with TLC (Temperature Controlled Logistics) may be informed about the requirement and proposed pick up point coordinated. Most couriers have an all India coordinator.

(b) Immediately after autopsy, the brain and muscle samples must be kept in the freezer compartment of the refrigerator and blood, urine, vitreous humour samples must be kept in the chill tray in the refrigerator till dispatch. Histopathology samples are to be kept at room temperature.

(c) The toxicology samples must then be transported in a vaccine carriers containing frozen gel/ice packs/dry ice and histopathology samples in a cardboard box by service aircraft to the nearest courier service pick up points. This should be accompanied by an escort preferably a Lab assistant/ Health assistant.

(d) At the courier pick up points, the labelled samples are repacked into specific validated boxes for 2-8°C (blood, urine and vitreous humour), frozen temp (brain and muscle) and room temperature (histopathology samples). Samples are to be sent by courier to Department of Aviation Pathology, Institute of Aerospace Medicine, Vimanapura Post Bangalore-17. A certificate required by the courier has to be signed by the medical officer and handed over with the sample.

(e) Prior coordination with the courier service should ensure that at no point the cold chain is broken. This will be specially required at additional pick up points (non TCL centres). Dispatch in vaccine carrier should occur from SMC only after the TCL boxes have reached the courier pick up points. It is preferable the IAM be informed telephonically with details of the dispatch.

Conclusion

The systematic collection, preservation & shipment of autopsy specimens is the first important step in any fatal aircraft accident investigation. The major amendments have been suggested to the present policy for specimen collection and transport keeping in view of the upcoming toxicology laboratory

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