Int J Legal Med (2012) 126:427–433
DOI 10.1007/s00414-012-0682-x

ORIGINAL ARTICLE

Nasal ciliary motility: a new tool in estimating the time of death

Maria Carolina Romanelli · Matteo Gelardi · Maria Luisa Fiorella · Lucia Tattoli · Giancarlo Di Vella · Biagio Solarino

Received: 13 September 2011 / Accepted: 14 February 2012 / Published online: 28 February 2012

Abstract Determination of time since death is one of the most difficult and crucial issue in forensic medicine. Apart from body cooling, which is commonly used in the early postmortem interval (PMI), supravital reactions are the most interesting postmortem changes for time of death estimation. Nasal ciliary motility has been occasionally observed in postmortem period although no studies have focused on this phenomenon for forensic purposes. We aimed to evaluate the diagnostic usefulness of ciliary motility as a potential tool in estimating the time of death. Specimens of ciliated epithelium from 100 consecutive cadavers were obtained by scraping the nasal mucosa at three different postmortem intervals. The samples were then smeared on a slide, and an in vitro evaluation of ciliary movement was analyzed by phase-contrast microscopy. A postmortem nasal ciliary motility was observed, and a statistically significant relationship between decreasing ciliary movements and increasing postmortem interval was detected even in presence of putrefactive changes of nasal ultrastructure integrity. Some peculiar causes of death seem to influence ciliary motility in the early PMI, while no significant correlations with sex or age were observed. According to the results of this study, postmortem evaluation of nasal ciliary motility may be a bona fide and a feasible option for estimating the time of death.

Keywords Nasal ciliary motility · Forensic medicine · Postmortem interval · Time of death

M. C. Romanelli · L. Tattoli · G. Di Vella · B. Solarino (✉)
Section of Legal Medicine, University of Bari,
Bari, Italy
e-mail: biagiosolarino@libero.it

M. Gelardi · M. L. Fiorella
Department of Otolaryngology, University of Bari,
Bari, Italy

Introduction

The estimation of the time since death (PMI, postmortem interval) is one of the most important points of debate in forensic practice since knowledge of PMI is essential in criminal investigation. Several studies have proposed diverse procedures for PMI determination but the election of the state-of-the-art procedure is still ongoing [1, 2].

Henßge developed a nomogram which is based on measurement of the body core temperature and also considered various parameters of environmental conditions (temperature, humidity, clothing, etc). This method is now well recognized and in widespread use in the early postmortem period [3–14].

Except for rectal temperature measurement, many studies have reported physiochemical approaches for PMI estimations. Supravital reactions of tissues are considered the practical and most efficient postmortem changes giving information on the time of death [15].

The irreversible circulatory arrest is the starting point for a period of survival of some tissues under a condition of global ischemia. The basis of such phenomenon is the anaerobic glycolytic metabolism, which tends to decrease inconstantly in each individual in the first 24 h [15]. For practical purposes in forensic medicine, most investigations on supravitality have been carried out on different morphological and functional levels: organ systems; isolated organs; tissues; and cells.

The nasal mucosa consists of an epithelium resting on a thin basal membrane that separates it from the tunica propria. The pseudostratified columnar epithelium is composed of ciliated and non-ciliated columnar cells. The latter are also called brush border or striate cells, muciparous goblet cells, and basal cells. All these cells are closely interconnected through desmoidal and hemidesmoidal junction systems. The ciliated cell is the most differentiated and numerous cell

type, accounting for about 80% of the cells making up the nasal mucosa. The ratio between ciliated and non-ciliated cells is 5:1 and increases proceeding distally toward the lower airways where it peaks at 100–200:1. Ciliated cells are elongated polygons, 15–20 μ high, with nuclei located at various heights above the basal membrane (Figs. 1 and 2).

The top surface is composed of about 100–250 cilia and about 300 microvilli. The cilia grasp a mucous gel layer (an important nonspecific defense system of the upper airways that decontaminates inhaled air) with their tips toward the nasopharynx where it is eliminated [16, 17].

Nasal scraping is the best method for obtaining a representative sample of nasal mucosa cells. It can be performed in clinical practice as a noninvasive procedure that causes the patient minor discomfort, and it is used in diagnosing primary and secondary ciliary dyskinesia, rare autosomal syndromes, and allergic rhinosinusitis. Usually, the cilia propel the gel layer in rhythmic movements at a mean frequency of about 800 beats per minute. Ciliary motility is determined by microtubule scrolls and is characterized by two different movement types: a rapid one called "propulsive movement" and a slower one called "return movement". Cilia flex synchronously with a frequency of 600–1,000 beats per minute, moving in the same direction, but with a slight phase difference defined "metachronous movement" [16].

A previous study conducted by Lee [18] on two cases showed normal ultrastructure integrity of the nasal cells that was maintained for at least 15 h post-mortem. Moreover, normal motility function was occasionally observed for as long as 18 h following death, thus pointing to ciliary motility as a clear sign of supravital reaction.

On these bases, in order to improve the reliability of the estimated PMI for forensic purposes, this work aims to study the variation of nasal ciliary motility in the early postmortem intervals.

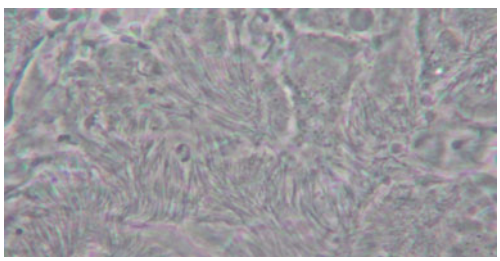

Fig. 1 Phase-contrast micrograph sample of pseudostratified columnar epithelium of the nasal mucosa. It is composed of non-ciliated and ciliated columnar cells. The ciliated cell is the most differentiated and numerous cell type, accounting for about 80% of the cells making up the nasal mucosa (5:1 ratio between ciliated and non-ciliated cells). ×40

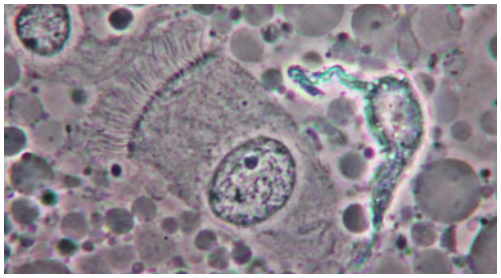

Fig. 2 Phase-contrast micrograph of a ciliated cell of the nasal mucosa. Ciliated cells are elongated polygons, 15–20 μ high, with nuclei located at various heights above the basal membrane. The ciliated cell is divided in three regions: apex with ciliary apparatus, body, and tail. ×100 in immersion

Material and methods

Study population and procedures

We prospectively studied 100 consecutive patients who died at University Hospital of Bari, from March to November 2010. In all cases, brain death was legally certified and then cadavers were admitted at the mortuary of local department of legal medicine within 2 h after death, waiting for autopsy or funeral. The bodies laid undressed on mortuary tables; local temperature varied from 17–18°C (March–April, October–November) to 22–26°C (May–September).

The age ranged from 23 to 98 years (average 71.7); the group was made up of 62 males and 38 females. All premortem information about the cause of death, including the medical treatments performed, were available in the Hospital file (Table 1). The time elapsed since death was known in all cases.

Table 1 The causes of death

Cause of death	Number (%)	Male/Female	Age (years) range
Natural deaths			
Cancer	23 (23)	15/8	25–84
Heart failure	14 (14)	7/7	38–98
Heart attack	15 (15)	11/4	28–97
Hepatic disease	3 (3)	1/2	52–70
Cerebral ictus	4 (4)	2/2	76–81
Septic shock	15 (15)	8/7	43–91
Ruptured aortic aneurysm	4 (4)	4/0	38–84
MOF	10 (10)	7/3	23–83
Respiratory failure	9 (9)	5/4	59–90
Traumatic deaths			
Burns	3 (3)	2/1	30–81

Methods and procedures

A specimen of ciliated epithelium was obtained by scraping the external portion of nasal mucosa with a spoon-shaped nasal probe (Rhinoprobe). Sampling sites were the inferior turbinate (central portion) or the middle turbinate. The samples were carried out in all cases at three different postmortem intervals ranging from 4 to 30 h: T1 (4–6 h, mean 5), T2 (10–12 h, mean 11), and T3 (>16 h, mean average of 25 h, maximum 30 h). The material picked up was then spread uniformly in the center of a slide, and after adding 1–2 drops of physiological solution at 36°C, it was covered by cover glass. After that, an in vitro evaluation of ciliary movement was performed: slides were analyzed by phase-contrast microscopy with ×100 objective lens in immersion. About 20 fields were observed for each slide, and once ciliary motility was recognized in some cells, those elements with a more active motility were counted and studied as time passed. Ciliary beat frequency (CBF) was recorded and classified as: present (3–4 beats/s), hypo-valid (1–2 beats/s), and absent. We also compared the ciliary beat frequencies with the causes of death, age, and sex.

Cases with bleeding nose or with tampons in nasal choanae were excluded from the study because scraping was not possible.

Statistical analysis

The comparison between multiple groups was assessed using one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison test as post hoc. All statistical calculations were performed with Excel (Office XP, Microsoft, Seattle, WA, USA) and Graph PadPrism software (version 5.01, Graph Pad Software, San Diego, CA, USA). P value is considered significant with $P<0.05$.

Results

Mucociliary motility and time of death

Nasal mucosa samples were obtained in all cases examined. Median time between death and scraping was 5 h at T1 (interval range between 4 and 6 h), 11 h at T2 (interval range between 10 and 12 h) and 25 h at T3 (interval range between 16 and 30 h). A comparison of ciliary motility outcome in the three different postmortem intervals was observed (Fig. 3).

In eighty cases in which samples were collected at T1, microscopic observation provided images of ciliary movements: 3–4/s CBF in 64 cases (64%) and 1–2/s CBF in 16 (16%). Ciliary activity could not be noted in the remaining 20 cases (20%).

Furthermore, in all these 20 samples in which ciliary motility was absent, microscopic observation and hospital file analysis provided additional information: six cases (30%) showed bacterial contamination of the sample, four (20%) had mycotic contamination, eight (40%) had medical records positive for chemotherapy, and two (10%) were negative for all the previous parameters.

Information about ciliary motility was obtained also during T2. Thirty-eight cases (38%) showed activity at 3–4/s CBF, 16 (16%) at 1–2/s CBF, whereas the remaining 46 samples (46%) didn't show any ciliary movements. The analysis of the cases with absent motility at T2 showed that at T1, 20 cases (44%) scored negative too, 14 (30%) showed 3–4/s CBF, and 12 cases (26%) 1–2/s CBF (Fig. 4).

When the observation period was longer than 16 h (T3), ciliary motility at 1–2 CBF was noted in only 17 (17%) of 100 cases, whereas all of the samples obtained from the remaining 83 cases (83%) showed absent activity. Results showed that the 17 cases with present ciliary movements corresponded to samples obtained when the postmortem interval was less than 21 h and that showed 3–4/s CBF at T1 and T2. The samples with absent motility were detected variably in a range between 16 and 30 h.

The analysis of ciliary motility outcome at T2 of the cases with absent CBF at T3 revealed that 21 cases (25%) showed 3–4/s CBF at T2, 16 cases (20%) with 1–2/s CBF, and 46 cases (55%) with no movements (Fig. 5).

The progressive observation of ciliary motility after death is indicative for a "natural" decrement of ciliary activity with 3–4/s CBF higher at T1 than at T2/T3; 0/s beat frequency rises with growing postmortem interval. The rate of hypo-valid motility is nearly constant with time. This trend can be explained by just observing that with increasing postmortem interval, some cases decrease in motility; therefore, we can speculate that some of the cases with 3–4/s CBF in the previous interval show 1–2/s CBF in the following one as well those with 1–2/s show absent motility in the subsequent interval. ANOVA followed by Newman–Keuls showed that ciliary motility rate was significantly lower at T3 than at T1/T2 ($P<0.0001$), suggesting that the postmortem interval was relevant in ciliary movement decrements (Fig. 6).

Correlation between ciliary motility and age

Linear regression did not show a significant difference in ciliary activity rate for all the three postmortem intervals between young and old patients (elderly patients presented 3–4/s CBF as well as the younger ones). Figure 7 represents

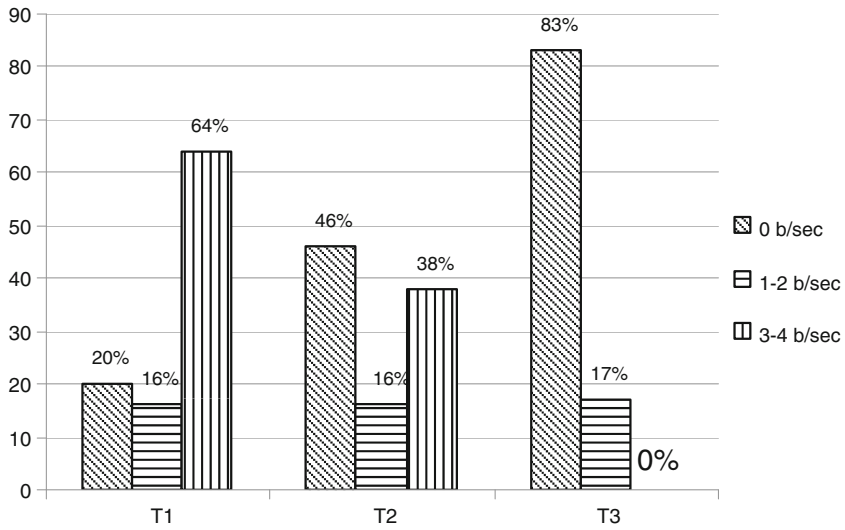

Fig. 3 Ciliary motility observed at T1, T2, and T3

ciliary motility at T1 in different age intervals, but data cannot be considered statistically valid, because the number of cases for age group is not comparable (Fig. 7).

Correlation between ciliary motility and sex

We performed student's *t* test and we found no significant changes between males and females in terms of mucociliary motility ($P<0.0892$).

Correlation between ciliary motility and cause of death

With regard to the cause of death, ciliary motility absence was frequently noted in cases with cancer, septic shock, and multiple organ failure (MOF), suggesting that infections and chemotherapy could play a role in ciliary movement impairment (Fig. 8).

Discussion

Hilding [19] has noted ciliary activity in the lower respiratory tract that continues 18 h post-mortem and is prolonged after storage. Earlier publication [18] has shown the evidence of postmortem ciliary movements that were occasionally observed in only two cases and more than 18 h after death.

In this study, we firstly confirmed that nasal ciliary motility continues after death and then we observed it tends to decrease with growing postmortem interval. Here, for the first time, we analyzed the progression of such phenomenon in the "early postmortem period" (usually defined as the time between death and the appearance of generalized putrefaction) and the results obtained raise the intriguing possibility to use the evaluation of such supravital reaction in estimating the time of death for forensic purposes.

The medicolegal evaluation of the time interval since death, given at an early stage, is of a paramount importance to the criminal investigators.

Several researchers have stressed the importance of supravitality reactions in comparison with body cooling measurement because these are not influenced by environmental factors. Most of these investigations have been carried out on skeletal muscle or smooth muscle (iris, arteries)

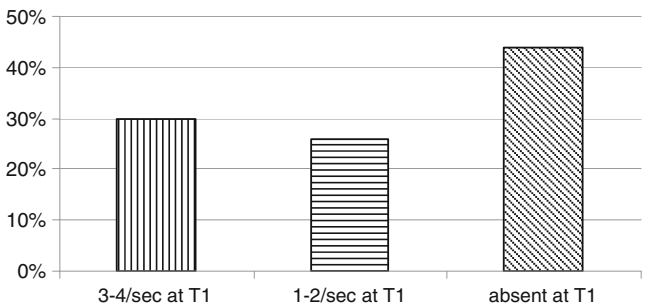

Fig. 4 Ciliary motility at T1 of the cases with absent CBF at T2

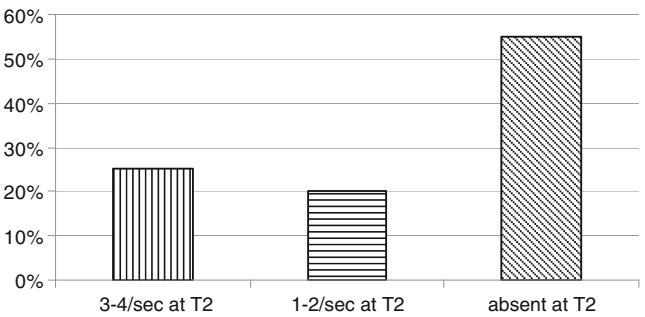

Fig. 5 Ciliary motility at T2 of the cases with absent CBF at T3

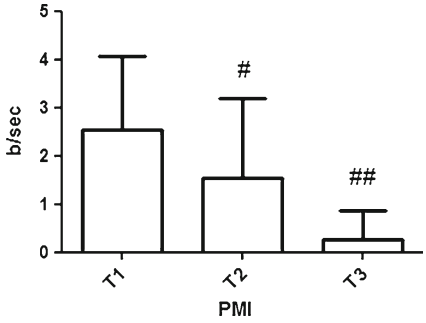

Fig. 6 Ciliary beat frequency at T1, T2, and T3. At T1, the majority of cases shows 3–4 b/s CBF more than in the other intervals with a constant decrement of motility rate ($P<0.0001$). The *single number sign* indicates significant difference vs T1; the double number sign indicates significant difference vs T1 and T2 (ANOVA followed by Newman–Keuls)

and evaluate the postmortem duration of excitability after electrical or pharmacological stimulation [20–22]. The Zsako's phenomenon is a mechanical excitation of muscle that can be seen in the first 2–3 h post-mortem, while idiomuscular contraction can be observed in the following hours [23, 24].

Postmortem transport of gastric content and spermatozoa viability are claimed to be spontaneous supravital activities even if definitive results regarding the time of death identification are still missing [25–27]. Thus, death time estimation using supravital reactions is considered useful and valuable from a theoretical viewpoint although rarely evaluated in everyday forensic practice. The reason is that most of these techniques, as well as new methods based on biochemical markers or other parameters [28–33], require some type of biopsy or collected biological fluids and laboratory tests. Moreover, there is the possibility of altering the crime scene. Finally, they are also time-consuming and cannot be performed in situ (except few of them), thus resulting in a delayed and less efficient investigation.

In our study, we investigated 100 cases in three postmortem intervals (T1–T3) within 30 h after death. Generally speaking 6, 12, and more than 16 h after death, the evaluation of body cooling, that is considered the most scientific sign in this field of evidence, shows an increasing range (of error) in time of death estimation. Our findings indicate that a statistical significant decrease of ciliary motility comparing with time elapsed since death exists. We observed that 3–4/s CBF rate is higher at T1 than at T2/T3. Moreover, 0/s CBF is correlated with growing postmortem interval. The rate of hypo-valid motility is nearly constant with time. Regarding this observation, we can speculate that some of the cases with higher CBF in the previous interval show a decrease of motility frequency in the following one since showing absent activity of the previous CBF was 1-2/s. In the present study, we then show that after 16 h in the early postmortem interval, there is a great probability to find absence of ciliary movements. Future studies might also include the observation of nasal ultrastructure integrity breakdown as well as the internal temperature measurement to better define the role of absence of ciliary motility in the definition of time death interval.

It is well known that cilia contain a central microtubular core called "axoneme" that forms a "9+2" arrangement where nine presents peripheral microtubule doublets (Fig. 9). These cilia beat in a rhythmic manner to propel mucus; the ATPase activity of the dynein arms generates the force required for ciliary beating and bending [34, 35]. On these bases, we can speculate that spontaneous ciliary activity after death depends on breakdown of ATP that influence the duration of this supravital phenomenon. Our present study indicates that after 16–18 h, most of energetic metabolism of cilia is ending even if the reason why normal ciliary motility in specific cases is maintained till 30 h, it is not known and deserves future investigations.

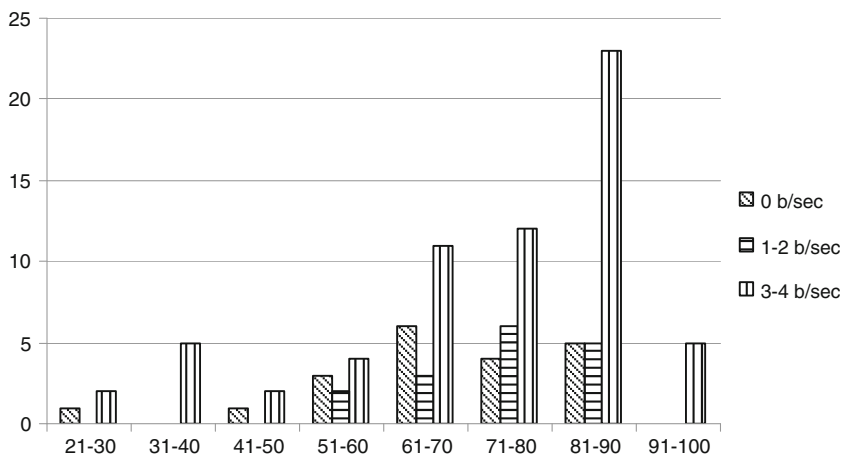

Fig. 7 Ciliary motility distribution at different age groups

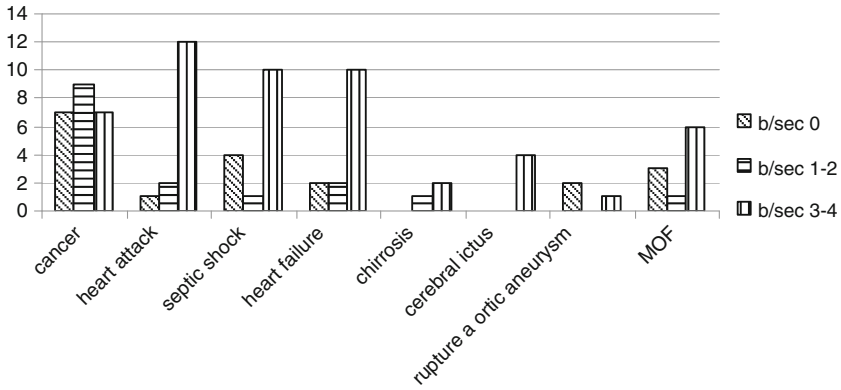

Fig. 8 Ciliary motility distribution compared with different cause of death groups

Lee [18] analyzed postmortem respiratory ciliary streaming and ultrastructure after placing epithelial samples of inferior turbinate in Krebs–Henseleit solution and then storing these specimens at 4°C for as long as 60 h after collection. We obtained nasal samples from fresh cadavers that were observed three times at least within 16 h post-mortem.

In living people, it is well recognized that measurement of ciliary beat frequency may be affected by environmental factors such as PH and temperature [36, 37]. Our results seem to show no difference in comparison with the season in which the samples were picked up. These data are obtained, indeed, just by observing the difference existing between the cases analyzed in June rather than in November, where morgue temperatures were evaluated in T1.

No significant correlation was observed between ciliary motility and sex; we can approximately exclude a decreasing activity in older people even if this data has no statistical relevance. According to international literature, reduction or absence of ciliary function are observed in congenital disease such as primary ciliary dyskinesia that is commonly associated with chronic sinusitis, bronchiectasis, sinus hypoplasia, secretory otitis media [38]. Other causes of ciliary beat frequency modification are polycystic hepatic and renal disease, allergic rhinosinusitis, and bacterial or fungal infections [36]. None of these diseases were specifically mentioned in the Hospital file record of the sample studied. Nevertheless, we noted an increasing absence of ciliary motility in people who died from cancer, sepsis, and MOF. This data suggested the possible role of the immune system in regulating such phenomenon even if further data are requested.

Our data support the idea that ciliary motility analysis may be a useful tool in evaluating the time of death in the early postmortem period and provide the impetus to study in the future this phenomenon in a large group of patients to better define the relationship between cause of death and time-lag presence of nasociliary motility. Further studies should indeed be undertaken to evaluate the effects of environmental factors (e.g., hypothermia), voluptuary factors (smoking, sniffing), and natural disease on ciliary beat activities.

Overall, in comparison with other supravital phenomena, nasal scraping is a very easy technique by which with a rhinoprobe, it is possible to keep a large part of ciliated epithelium. This method is non invasive, inexpensive, and with a basic microscope, it is possible to observe the supravital reaction shortly after scraping without delay that is normally required to analyze other markers.

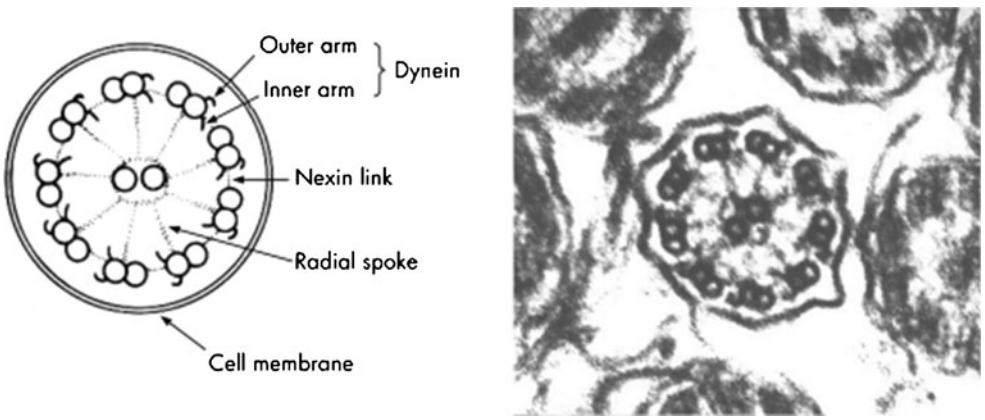

Fig. 9 Schematic diagram and electron micrograph of a ciliary ultrastructure: cilia are composed of an array of nine double microtubules arranged in a peripheral ring surrounding a central pair (9+2). ×50,000

Acknowledgments We wish to thank Dr. Antonio Moschetta for his critical review of the manuscript.

References

1. Lundquist F (1956) Physical and chemical methods for the estimation of the time of death. Acta Med Leg Sog Liege 9:205–213
2. Madea B (2005) Is there recent progress in the estimation of the postmortem interval by means of thanatochemistry? Forensic Sci Int 151:139–149
3. Marshall TK, Hoare FE (1962) Estimating the time of death—the rectal cooling after death and its mathematical expression. J Forensic Sci 7(1):56–81
4. Henßge C (1981) Todeszeitschätzungen durch die mathematische Beschreibung der rektalen Leichenabkühlung unter verschiedenen Abkühlbedingungen. Z Rechtsmed 87:147–178
5. Henßge C, Brinkmann B, Püschel K (1984) Todeszeitbestimmung durch Rektaltemperaturmessungen bei Wassersuspension der Leiche. Z Rechtsmed 92:255–276
6. Henßge C, Althaus L, Bolt J, Freislederer A, Haffner HT, Henßge CA, Hoppe B, Schneider V (2000) Experiences with a compound method for estimating the time since death. I. Rectal temperature nomogram for time since death. Int J Leg Med 113(6):303–319
7. Henßge C, Althaus L, Bolt J, Freislederer A, Haffner HT, Henßge CA, Hoppe B, Schneider V (2000) Experiences with a compound method for estimating the time since death. II. Integration of non-temperature-based methods. Int J Leg Med 113(6):320–331
8. Henßge C, Madea B (2004) Estimation of the time since death in the early postmortem period. Forensic Sci Int 144:167–175
9. Henßge C, Knight B, Krompecher T, Madea B, Nokes L (2002) The estimation of the time since death in the early postmortem period. Edward Arnold, London
10. Muggenthaler H, Sinicina I, Hubig M, Mall G (2011) Database of post-mortem rectal cooling cases under strictly controlled conditions: a useful tool in death time estimation. Int J Leg Med 126 (1):79–87
11. Hubig M, Muggenthaler H, Mall G (2011) Influence of measurement errors on temperature-based death time determination. Int J Leg Med 125(4):503–517
12. Henßge C (1988) Death time estimation in case work. I. The rectal temperature time of death nomogram. Forensic Sci Int 38:209–236
13. Henßge C (1992) Rectal temperature time of death nomogram: dependence of corrective factors on the body weight under stronger thermic insulation conditions. Forensic Sci Int 54:51–56
14. Zhou C, Byard RW (2011) Factors and processes causing accelerated decomposition in human cadavers—an overview. J For Leg Med 18:6–9
15. Madea B (1994) Importance of supravitality in forensic medicine. Forensic Sci Int 69:221–241
16. Gelardi M (2007) Atlas of nasal cytology for the differential diagnosis of nasal diseases. Centro Scientifico Editore, Torino 21–26 e 16–17
17. Caruso G, Gelardi M, Passali G, De Santi M (2007) Nasal scraping in diagnosing ciliary dyskinesia. Am J Rhinol 21(6):702–705
18. Lee R, Rossman C, O'brodovich H (1987) Assessment of postmortem respiratory ciliary motility and ultrastructure. Am Rev Respir Dis 136:445–447
19. Hilding AC (1957) Ciliary streaming in the lower respiratory tract. Am J Physiol 191:404–410
20. Mayer M, Neufeld B (1980) Post-mortem changes in skeletal muscle protease and creatine phosphokinase activity—a possible marker for determination of time of death. Forensic Sci Int 15 (3):197–203
21. Madea B, Henßge C (1990) Electrical excitability of skeletal muscle postmortem in case work. Forensic Sci Int 47(3):207–227
22. Püschel K, Brinkmann B, Lieske K (1985) Ultrastructural alterations of skeletal muscles after electric shock. Am J Forensic Med Pathol 6(4):296–300
23. Madea B (2002) Post-mortem mechanical excitation of skeletal muscle. In: Henßge C, Knight B, Krompecher T, Madea B, Nokes L (eds) The estimation of the time since death in the early postmortem period. Edward Arnold, London, pp 160–164
24. Madea B (2002) Post-mortem electrical excitability of skeletal muscle in case-work. In: Henßge C, Knight B, Krompecher T, Madea B, Nokes L (eds) The estimation of the time since death in the early post-mortem period. Edward Arnold, London, pp 164–206
25. Madea B, Oehmichen M, Henßge C (1986) Postmortem transport of gastric contents? Z Rechtsmed 97(3):201–206
26. Jaffe FA (1989) Stomach contents and the time of death. Reexamination of a persistent question. Am J Forensic Med Pathol 10 (1):37–41
27. Collins KA, Bennett AT (2001) Persistence of spermatozoa and prostatic acid phosphatase in specimens from deceased individuals during varied post-mortem intervals. Am J Forensic Med Pathol 22 (3):228–232
28. Baccino E, De Saint ML, Schuliar Y, Guilloteau P, Le Rhun M, Morin JF, Leglise D, Amice J (1996) Outer ear temperature and time of death. Forensic Sci Int 83:133–146
29. Cattaneo C, Di Giancamillo A, Campari O, Martrille L, Jouineau C (2009) Infrared tympanic thermography as a substitute for a probe in the evaluation of ear temperature for postmortem interval determination: a pilot study. J Forensic Leg Med 16:215–217
30. Jashnani KD, Kale SA, Rupani AB (2010) Vitreous humor: biochemical constituents in estimation of postmortem interval. J Forensic Sci 55(6):1523–1527
31. Estracanholli ES, Kurachi C, Vicente JR, Campos De Menezes PF, Castro O, Junior S, Bagnato VS (2009) Determination of postmortem interval using in situ optical fluorescence. Opt Express 17 (10):8185–8192
32. Singh D, Prashad R, Sharma S, Pandey AN (2006) Estimation of post-mortem interval from human pericardial fluid electrolytes concentrations in Chandigarh zone of India: log transformed linear regression model. Leg Med 8:279–287
33. Yadav J, Deshpande A, Arora A, Athawal BK, Dubey BP (2007) Estimation of time since death from CSF electrolyte concentration in Bhopal region of central India. Leg Med 9:309–313
34. Bush A, Chodhari R, Collins N, Copeland F, Hall P, Harcourt J, Hariri M, Hogg C, Lucas J, Mitchison HM, O'Callaghan C, Phillips G (2007) Primary ciliary dyskinesia: current state of the art. Arch Dis Child 92(12):1136–1140
35. Berbari NF, O'Connor AK, Haycraft CJ, Yoder BK (2009) The primary cilium as a complex signaling center. Curr Biol 19(13): R526–R535
36. Thomas B, Rutman A, O'Callaghan C (2009) Disrupted ciliated epithelium shows ciliary beat frequency and increased dyskinesia. Eur Respirt J 34:401–404
37. Clary-Meinesz CF, Cosson J, Huitorel P, Blaive B (1992) Temperature effect on the ciliary beat frequency of human nasal and tracheal ciliated cells. Biol Cell 76(3):335–338
38. Armengot M, Milara J, Mata M, Carda C, Cortijo J (2010) Cilia motility and structure in primary and secondary ciliary dyskinesia. Am J Rhinol Allergy 24(3):175–180