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THE ROLE OF ^{14}C DATING IN THE IDENTIFICATION OF MISSING PERSONS IN CYPRUS

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ABSTRACT. The Committee on Missing Persons in Cyprus (CMP) is a bicommunal committee with the mandate to locate and identify the skeletal remains of 2002 persons who were reported missing during the inter-communal fighting of 1963–64, as well as the events of July and August 1974. During the periods of conflict, several archaeological sites and old cemeteries were used as primary burial sites, among several other types of burials, as they were easily accessed by the persons involved in the interment and little effort was needed to conceal the bodies. The relatively large post-mortem interval and the generally poor post-mortem preservation of the skeletal remains poses an additional challenge in the forensic examination process and the identification of the remains, particularly in the absence of a context or other associated artifacts/evidence. Between 2016 and 2020, the CMP has been collaborating with CEDAD to clarify the relevancy of several cases by using radiocarbon dating. The CMP submitted 139 cases to CEDAD out of which 112 were determined as not linked to the 1963–64 and 1974 events and then not relevant for the CMP project. For the remaining samples radiocarbon dating was used to determine death age.

KEYWORDS: bomb peak dating, forensics, human remains identification, radiocarbon.

INTRODUCTION

Radiocarbon dating is applied in the forensics practice in different areas including the analysis of skeletal remains (Handlos et al. 2018; Hajdas et al. 2021), the analysis of drugs of abuse (Zoppi et al. 2004), of foodstuff (Chytry et al. 2022; Quarta et al. 2022), and the fight against the illegal trade of materials obtained from endangered species (Quarta et al. 2019; Wild et al. 2019). In these applications the "bomb peak", the excess in the atmospheric radiocarbon concentration associated with aboveground nuclear detonation tests, is used to achieve chronological resolution of a few years for samples younger than seventy years. Indeed, different studies have shown the potential of the approach and recently also the IAEA (International Atomic Energy Agency) has supported a Coordinated Research Program aimed at enhancing the use of nuclear-based techniques, then including AMS ^{14}C-dating, in the routine forensics practice, bridging the gap between AMS experts and forensics stakeholders (Quarta et al. 2022). In particular, in forensic anthropology ^{14}C dating is nowadays widely used to obtain information about the forensics relevance of human remains, the death and birth ages, the age at death supplying often crucial information in the identification of missing persons. In this paper, we report on the analysis carried out on the remains, typically bones, recovered in the island of Cyprus by the Committee on Missing Persons (CMP). The aim of the study was to support the CMP Project on the identification of more than two thousand persons (492 Turkish and 1570 Greek Cypriots) who went missing during the two waves of violence which hit Cyprus in the 1960s and 1970s. We show how radiocarbon dating was extremely effective in assessing the relevance of the investigated bones for the purposes of the CMP. By

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reporting on three cases, we also show how the interpretation of ^{14}C data requires careful analysis of the results and a knowledge of the issue related to complex aspects such as carbon turnover in the different analyzed tissues.

THE CMP PROGRAM IN CYPRUS

Two waves of violence hit Cyprus starting in the 1960s. The first period broke out between the Greek Cypriot and Turkish Cypriot communities in 1963–64, leading to the disappearances of approximately 270 persons. A second wave of violence broke out in the summer of 1974, which led to the disappearance of approximately 1750 persons and to the de facto division of the island. The fighting in 1963–64 and the events of 1974 left the island in disorder and mourning for the missing. The Greek Cypriot and Turkish Cypriot communities conducted a series of talks in the following years which resulted in 1981 in the establishment of the Committee on Missing Persons in Cyprus (CMP) with the auspices and the support of the United Nations (Mikellide 2014).

The primary objective of this project is to enable relatives of missing persons to receive the remains of their loved ones, arrange for a proper burial and close a long period of uncertainty. The mandate of the CMP is purely humanitarian and does not attempt to establish the cause of death or attribute in any way responsibilities for the death of missing persons. The CMP employs a forensic team of more than sixty Cypriot archaeologists, anthropologists and geneticists, who conduct excavations throughout the island and carry out anthropological analysis and interpret and confirm the genetic results of the remains (Mikellide 2017; Ktori and Baranhan 2018).

The CMP Identification Process

The identification process carried out by the CMP is a complex procedure, established on the basis of the best international practices. Up to date, 1027 Missing Persons have been identified and returned to their families for proper burial. A detailed description of all the phases in which the entire procedure is structured is beyond the scopes of this paper, but in summary, it includes six main phases which are: investigation phase, archaeological phase, anthropological phase, genetic phase, reconciliation phase and finally identification and return of the remains Phase (Zorba et al. 2020). It is worth underlining here that the team of experts has often had to face complex cases as the result of complex and stratified burial contexts, the absence of context information or artifacts associated with the rests (such as personal belongings and clothes), and sometimes the mixing of the remains with archaeological or ancient material. The main objective of the anthropological analysis is then to obtain from the available remains as much information as possible. This phase includes the determination of the origin of the samples (human or non-human), the sorting of commingling, and eventually the number of individuals represented in the skeletal assemblage, the establishment of the biological profile of the individual (sex, age at death, stature), and obtaining all possible information helpful for the identification of the remains (ante and peri-mortem trauma, pathologies and dental information). In particular, one of the most relevant outcomes expected from the anthropological phase is the confirmation that the remains are relevant to the CMP's mandate and that they are potentially belonging to persons who went missing during the 1960s and 1970s events.

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The Role of ^{14}C Dating

In this project the role of ^{14}C dating is to support the anthropologists and to assess the antiquity of the remains, establishing whether the analyzed remains belong to individuals who died in 1963/4 or in 1974. ^{14}C dating analyses are either a preliminary, screening step preceding other analyses such as the DNA analysis or they can follow unsuccessful DNA matching to assess the age of the samples, and then eventually attribute the samples to the 1963/4 or 1974 events. Unfortunately, as sometimes happens in forensic cases, straight answers do not always correspond to easy questions. Indeed, depending on the measured ^{14}C content and age, different situations can happen. The first possibility is that a radiocarbon age older than ~270 years ($F^{14}C<0.97$) is measured. When this happens, ages can be calibrated to periods before the beginning of the 18th century, allowing to rule out cases as being relevant to the CMP mandate. As we will see this is, indeed, happening for the majority of the submitted samples. When radiocarbon concentration higher than ~0.97 are measured, a more careful analysis of the data is needed because the forensic interest cannot be excluded *a priori*. Indeed, the interpretation of these data requires considerations about the carbon fixation time and turnover in the living tissues and then in the analyzed bones. Another aspect is related to the proper placement of the measured ^{14}C concentration on the bomb curve and then two possible dates are typically obtained. that Several studies have demonstrated that (radio)carbon turnover in bones is significantly slower than in other tissues such as hair, nails, blood and most of the soft tissues (Ubelaker and Parra 2011). This results in a significant difference between the ^{14}C concentration measured in bones and the atmospheric value in the year of birth or death. In other words, in tissues with slow turnover rates such as bones, radiocarbon atmospheric values are relatively slowly incorporated through the food chain so that the time difference between atmospheric and concentration of radiocarbon into the analyzed tissues can be of the order of several years (Ubelaker 2014). This is shown in Figure 1 reporting, as a function of the age at death, the difference between the apparent age determined by ^{14}C dating bone collagen and the real age of death of an individual (Wild et al. 2000; Ubelaker et al. 2005; Hedges et al. 2007; Hodgins 2009; Calcagnile et al. 2013; Ubelaker et al. 2022). This effect is explained as due to the continuous uptake of carbon, with different ^{14}C signatures, during the lifetime of an individual with turnover rates which are decreasing from childhood to adulthood, reaching a steady state renewal speed until death.

Despite the large scattering of the data, some qualitative features can be extracted from Figure 1. For young individuals (age <20 years), the time lag is typically low and corresponds to a few years. This time lag increases with the increasing age at death becoming >25/30 years for individual older than ~40 years. Indeed, Ubelaker et al. (2015) found that the lag time is minimal ~3 years for individuals between 10 and 19 years of age, increasing to 25 years for individual between 50 and 59 years of age, reaching ~31 years for elderly individuals. The robustness of the age of death estimation is then highly correlated to the knowledge of the age at death of the analyzed individual, and then, in our case, to the information obtained during the anthropological examination of the remains. More recently statistically significant differences in the time lag measured for different bones (anterior midshaft femur, occipital, parietal, and vertebral body) of the same individual as well as large variability among different individuals with the same age have been also demonstrated (Ubelaker et al. 2022).

Interpretation of ^{14}C Dating: Carbon Turnover Modeling

In order to interpret the ^{14}C dating results by taking into account the abovementioned effects, the ^{14}C concentration in the analyzed tissue F(t) at time t was expressed as:

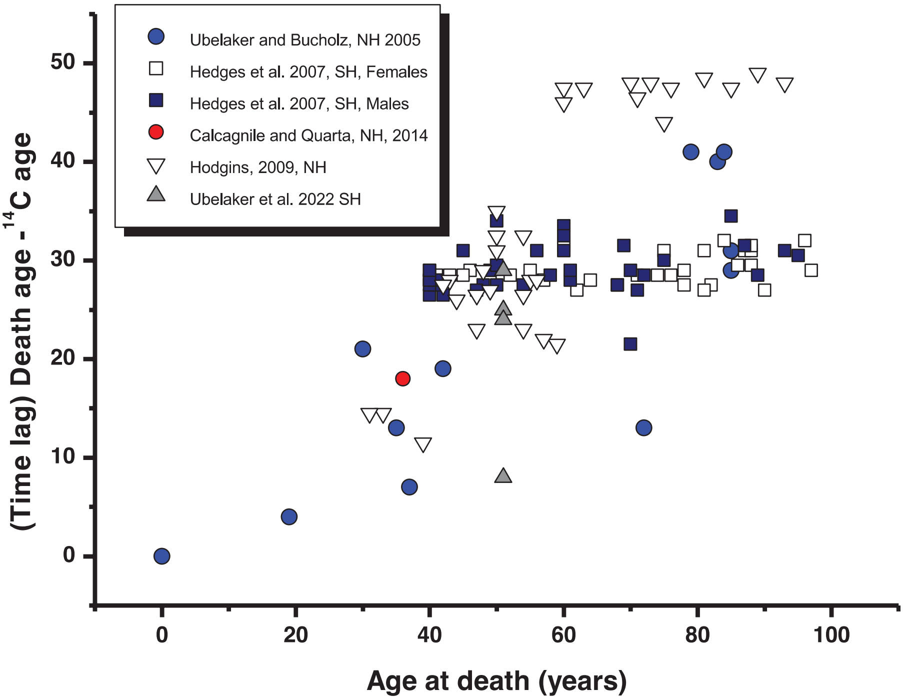

Figure 1 Collection of literature data about the time-lag between the real death age and the measured ^{14}C age as determined through the dating of bone collagen and the age at death of the dated individual.

$$F(t) = F(t - \Delta t) \cdot \left(1 - e^{-\frac{1}{r}}\right) + F_{atm}(t) \cdot e^{-\frac{1}{r}}$$

where: $F(t - \Delta t)$ is the ^{14}C concentration in the tissue at the time $t - \Delta t$, Δt is the set time step, $F_{atm}(t)$ is the ^{14}C concentration at the time t in the atmosphere which was obtained by refereeing to the IntCal2020 and the Northern hemisphere post-bomb data (Hua et al. 2021), $r = \frac{\Delta m}{m}$ is the carbon exchange rate.

The dependence of the carbon exchange rate on the age of the individual was taken into account by considering the r term to be variable with time. In particular, the age dependent turnover model as suggested by Hedges et al. (2007) was used.

Interpretation of ^{14}C Dating: Dietary Effects

Another aspect to be properly taken into account in the interpretation of ^{14}C data is related to the possible contribution into the diet of the dated individuals from marine carbon sources. Indeed, this cannot be ruled out a priori also considering the fact that Cyprus is an island. A high proportion of marine food into the diet would results in a reduced ^{14}C concentration and then in apparently older ^{14}C ages. In order to exclude this effect, the carbon and nitrogen stable isotopic ratios (expressed as $\delta^{13}C$ and $\delta^{15}N$) were measured on the collagen extracted from the samples. In fact, the consumption of marine food would also be recorded as an increase in the carbon and nitrogen stable isotopic ratios (Hedges et al. 2007).

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MATERIALS AND METHODS

Radiocarbon dating analyses were performed at CEDAD (Centre of Applied Physics, Dating and Diagnostics) at the Department of Mathematics and Physics "Ennio de Giorgi"-University of Salento on 139 bone samples submitted by the CMP between 2017 and 2020. Particular care was taken in the preservation of the chain of custody of the samples. No information about the submitted cases was supplied at the initial stage to the laboratory.

AMS analyses were carried out by using the AMS-^{14}C beamline based on a 3 MV Tandetron accelerator (Model HVEE 4130 HC) (Calcagnile et al. 2005, 2019). The samples were processed according to standard procedures as detailed in D'Elia et al. (2004). In particular, all the samples were observed at the optical microscope in order to highlight and mechanically remove possible macro-contaminants. Information about the preservation status of the samples was obtained through the measurement of the C/N ratio (van Klinken 1999). This consisted in the extraction of collagen which was performed by a following the Longin (1971) protocol modified as detailed in Quarta et al. 2004. The extracted collagen was then dried at 60°C and sealed in pre-evacuated quartz tubes ($< 10^{-4}$ mbar) together with silver wool and copper oxide. The samples were then combusted at 900°C for 4 hr and the released CO_2 recovered and cryogenically purified (D'Elia et al. 2004). The purified carbon dioxide was then reduce to solid graphite at 600°C by using high purity H_2 as reductant and Fe powder as catalyst. Reaction water was removed during the process by a water trap kept at –40°C with a cold finger. Carbon isotopic ratios were measured with the AMS system by comparing the beam currents (for ^{12}C and ^{13}C stable isotopes) and the ^{14}C ionization events in the GIC (Gas Ionization Chamber) obtained for the samples with those obtained with IAEA C6 sucrose standards used as reference. The measured ^{14}C/^{12}C isotopic ratios were, after correction for isotopic fractionation and processing and machine blank, used to calculate the conventional radiocarbon ages or the radiocarbon concentrations according to Stuiver and Polach (1977). Carbon and nitrogen stable isotopic ratios were measured by IRMS (isotope ratio mass spectrometry) at CEDAD by using a Delta V Plus spectrometer connected to a Mod. Flash 2000HT Elementar Analyzer both by Thermo. The Elementar Analyzer was also used to measure the C and N content of the sample and then the C/N ration used to assess the quality of the extracted collagen (Maruccio et al. 2017).

RESULTS

The radiocarbon concentrations measured for all the samples are shown in Figure 2. It can be seen that the majority of the samples (77) produced conventional radiocarbon ages >270 yr BP and ranging between 293 ± 40 yr BP (F^{14}C=0.9641 ± 0.0047) and 2953 ± 40 yr BP (F^{14}C=0.6923 ± 0.0038). All these samples were then labeled as "ancient" and not relevant to the CMP mandate. In 35 samples a radiocarbon age between 23 ± 45 and 261 ± 45 yr BP was measured and these samples were labeled as "pre-bomb" while in the remaining 16 sample the excess of ^{14}C corresponding to nuclear detonation tests was detected. For these "post-bomb" samples, F^{14}C values ranging between 1.0049 ± 0.0043 and 1.3526 ± 0.0057 were measured. For each of the "pre-bomb" and "post-bomb" sample, a detailed interpretation of the radiocarbon results was performed but a possible interest for CMP was not immediately excluded. Eleven samples failed to give results because of poor collagen preservation.

Of course, the discussion of each of the case is not possible within this paper, but we report on the detailed description of three cases which can be considered examples of the different situations, of the different ^{14}C results and of the complexity of data interpretation. The results obtained for these cases are summarized in Table 1. The C/N ration vary between 3.1 and 3.3

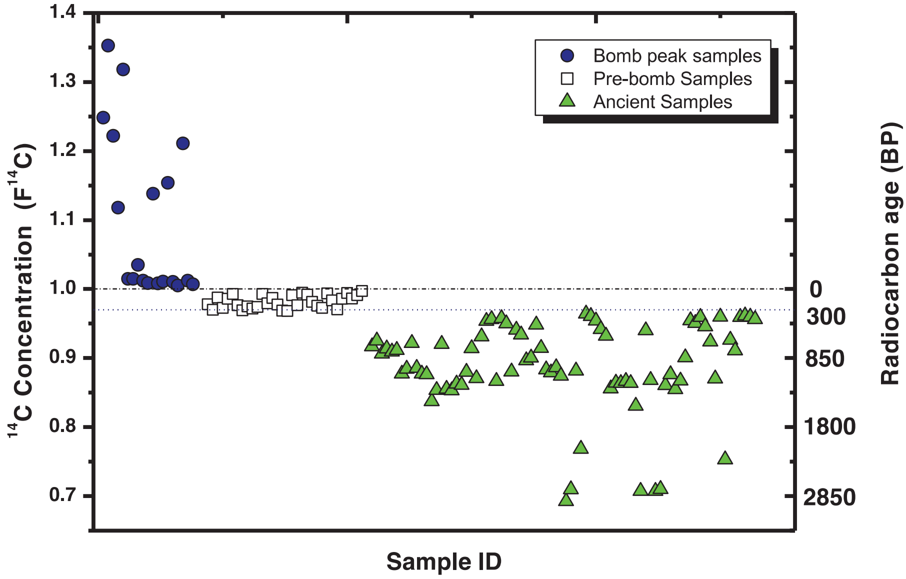

Figure 2 Measured radiocarbon concentration (left vertical axis) and conventional radiocarbon age (right vertical axis) for the analyzed samples.

and are then well within optimal range (2.9–3.6) indicating good collagen preservation and the absence of post-depositional changes (van Klinken 1999). Stable carbon and nitrogen IRMS data are indicative or a terrestrial diet allowing to exclude any dietary offset in the measured ^{14}C concentrations.

Case Study 1

The first case refers to human remains found by the workers during a road construction in a village. The excavation by the CMP archaeologists was conducted between December 2009 and January 2010. An incomplete skeleton was recovered, and it was transferred to the CMP Anthropological Laboratory.

The anthropological examination of the remains indicated that they belonged to a male individual, over 50 years old. Osteo-arthritis and age-related degenerative changes were observed on the skeleton. No peri-mortem traumas were observed on the skeleton and no associated clothing or artifacts were recovered with the remains. Following the anthropological analysis, a right femur section was cut and used for DNA analysis for identification purposes; however, the DNA profile from the bone sample did not match with any entry in the Family References Samples Database.

To assess whether the case was relevant to the CMP mandate or not, the same femur was sampled and submitted to radiocarbon analysis. In particular, the cortical bone of the midshaft of the femur was used for the analyses. A radiocarbon age of 346 ± 45 yr BP was measured corresponding to a calibrated age ranging between 1472 and 1636 AD with a probability of 95.4% (Reimer et al. 2020). The sample was then considered “ancient” and the corresponding case was closed as not relevant to the CMP Project.

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Table 1 Summary of the analysed sample, the selected tissue, carbon and nitrogen isotopic ratios, C/N ratio and measured ^{14}C concentration. Carbon and nitrogen IRMS data are reported relative to the internationally accepted standards V-PDB and AIR.

Ref	Sample	Lab code	Sample fraction	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N	$F^{14}C \pm 1\sigma$
Case 1	C14017	LTL17080	Femur midshaft Cortical bone	–18.8	11.1	3.1	0.9578 ± 0.0053
Case 2	C14042	LTL17105	Parietal bone	–17.9	11.3	3.2	1.0108 ± 0.0047
Case 3	2020-C14118	LTL20664	Humerus midshaft Cortical bone	–18.6	11.0	3.2	1.2109 ± 0.0041

Case Study 2

The second case refers to a more complex situation both in terms of the contextual information and ^{14}C data interpretation. According to witness information, a skull belonging to a soldier was removed from its initial burial site and thrown into a pit inside an old cemetery. The primary burial site was excavated in 2012 in an open field by the CMP and commingled remains belonging to at least two (2) individuals were recovered from the site. The exhumation of the skull was conducted in the cemetery in 2013. It was recovered at 35 cm depth together with other bones which did not associate together.

The anthropological examination of the remains indicated that the skull belonged to elderly (>60 years old) and edentulous male individual, while the DNA analysis performed on eight (8) skeletal samples from the original burial showed a minimum number of five (5) individuals that did not match with any of the family reference samples of the missing persons. In addition, the genetic analysis did not show any association between the remains found in the two different burial sites (the open field and the cemetery). A sample from the parietal bone of the skull was used for radiocarbon dating. In this case a radiocarbon concentration of $F^{14}C=1.0108\pm0.0047$ was measured, corresponding to a post-bomb sample.

In order to assess whether the case was relevant for the CMP mandate, we used the model for carbon turnover described above to simulate the expected ^{14}C concentration into the tissue for an elderly male dead either in 1964 or 1974. In particular, the model was tuned by using the data on parietal bones measured in Ubelaker et al. (2022).

Figure 3 shows the simulation of the expected ^{14}C concentration in the parietal bone tissues in the case of a 60-year-old man who died either in 1964 (and then born in 1904) or 1974 (and then born in 1914). The results indicate the expected radiocarbon concentration for an individual born in 1904 or 1914 would be 1.07 or 1.25 $F^{14}C$, significantly higher than the measured value of 1.01. We can then conclude that the skull is not relevant for the CMP project.

Case Study 3

According to witness information, one individual had been buried close to a water tank in an open field. An excavation was conducted in September 2007 and the remains of one individual were recovered from the site and were transferred to the CMP Anthropological Laboratory.

The anthropological examination of the remains indicated that the remains consisted of the left humerus and three long bone fragments with no signs of peri-mortem trauma. Concerning age at death, the scarcity of the available material only allowed to estimate an age >15 years. After the anthropological analysis was completed, a section of the left humerus was submitted for DNA analysis for identification purposes. The DNA profile from the bone sample did not match with any entry in Family References Samples Database. To clarify whether the case was relevant to the project, the midshaft of the left humerus was sampled and the cortical bone fraction submitted for radiocarbon analysis. In this case, a post-bomb radiocarbon concentration of $F^{14}C=1.2109 \pm 0.0041$.

Unfortunately, in this case the information about the age at death was very poor and we could not follow the same approach used for case 2. We then modeled the expected radiocarbon concentration for a person who died either in 1964 or 1974 which allowed us to reconstruct the ^{14}C concentration as a function of the age at death (Figure 4). For a person dead in 1964, the

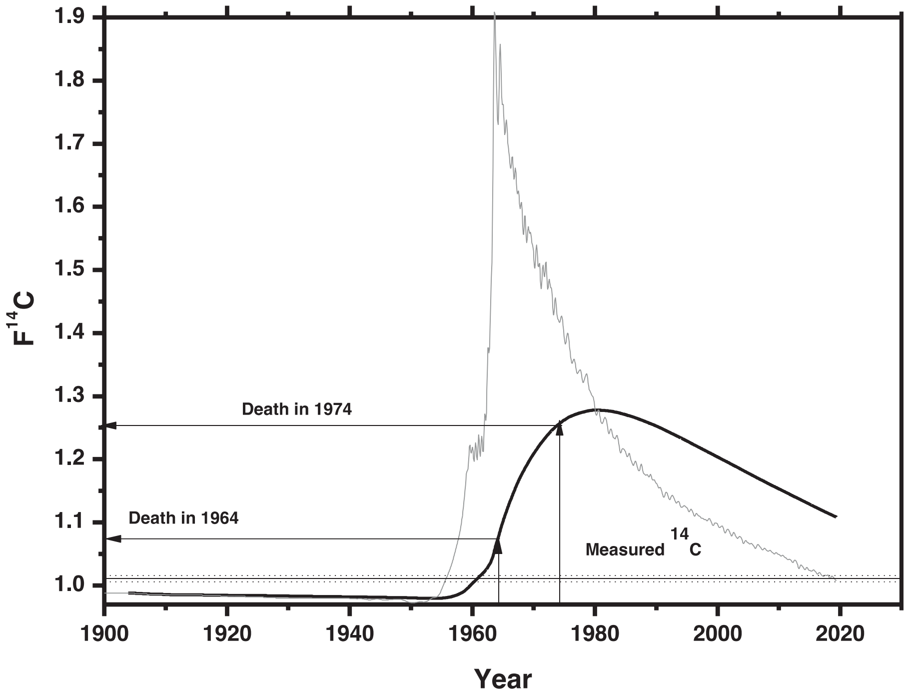

Figure 3 Results of the analysis carried out on the skull of case 2. The Northern Hemisphere bomb atmospheric concentration is given as a reference (gray curve) as obtained by merging the bomb peak data by Hua et al. (2021) and the IntCal20 curve. The modeled ^{14}C concentration is then shown for the two cases of an individual died at 60 years of age in 1964 or 1974 (the curve obtained in the two cases overlap).

measured ^{14}C concentration would be compatible with a child, though this possibility seems to be excluded by a determined age at death >15 years. The second possibility is that of a young adult (15–20 years of age) who had died in 1974. Consequently, in this case it is not possible to draw any conclusion about the relevance of the case to the CMP mandate and the investigation is still ongoing.

CONCLUSIONS

AMS radiocarbon analyses were carried out on human bones recovered by the CMP in Cyprus within the efforts to recover and identify more than 2000 persons who went missing during the events of 1963–64 and 1974. The aim of the analysis was to establish whether the bone samples were within the CMP mandate or not. The potential of radiocarbon dating in this field has been shown, together with the complexity associated with the interpretation of results as resulting from carbon turnover in the different tissues. Radiocarbon dating allowed to obtain conclusive results in most of the cases, for instance those which could be identified as "ancient", or in those cases where additional information (such as the age at death) where available from the anthropological analysis.

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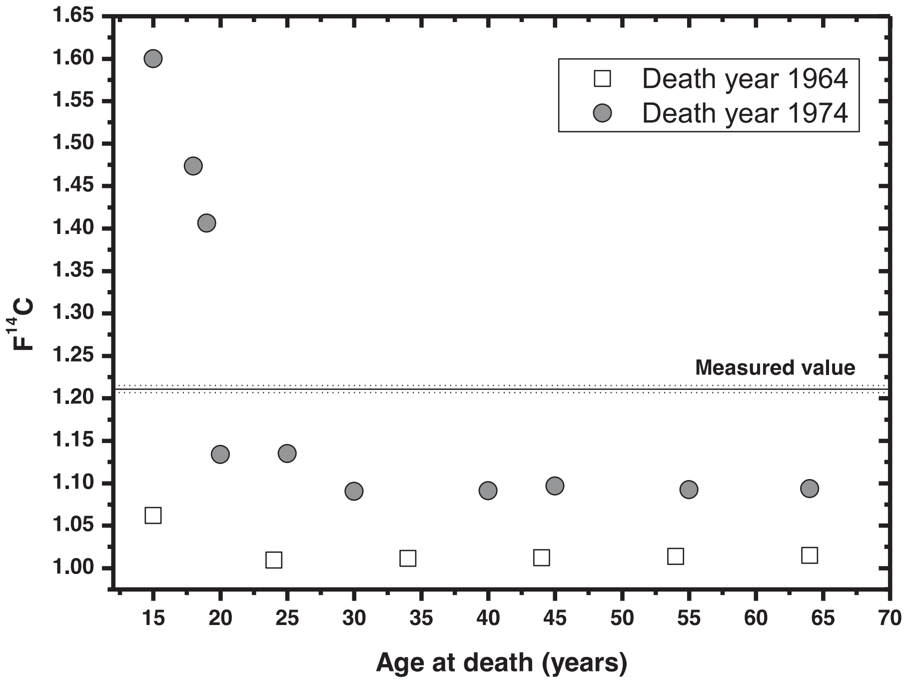

Figure 4 Modeled ^{14}C concentration for the cortical bone of an individual died either in 1964 or 1974 as a function of the age at death.

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