ABSTRACT

Carbon monoxide (CO) poisoning is a common cause of death and a public health problem. The authors studied the epidemiology and the *postmortem* forensic aspects in all the cases with a carboxihemoglobin (COHb) analysis, by reviewing the reports of all the autopsies performed in the Forensic Pathology Department of the Centre Branch of the National Institute of Legal Medicine of Portugal, between 2000 and 2010. The following criteria were analyzed: year, month, season of the year, age, gender, occupation, underlying disease, etiology, source of CO, cause of death, autopsy findings and toxicological analysis results. The relevance of the circumstantial information and the autopsy findings, the evolution during the years of CO poisoning as a cause of death and the challenges of its differential diagnosis, in our practice, are discussed.

Between January 2000 and December 2010, 69 COHb analysis were requested in our institution. In approximately 70% of the situations, circumstantial information included a source of CO at the death scene. More than a half of the cases presented thermal lesions. Cherry-red lividity and cherry-red coloration of blood and viscera were found in approximately 30% of the cases. In the studied period, 14 cases (20.3% of all requests) were recorded as CO poisonings. The highest number of cases occurred in 2000 and, for the remainder of the decade, years with no cases followed lower peaks. Winter registered the highest frequency of CO poisonings (53.8%), more frequent in males (69.2%) and in age group of 51-60 year olds (3 cases). 69.2% of the CO poisonings were violent, unintentional deaths and the remainder of the cases were suicides. Fire was the most frequent source of CO (5 cases, 38.5%). Cherry-red lividity was present in 8 (61.5%) CO poisonings and all presented cherry-red blood and viscera coloration, which was related to higher COHb levels (58 ± 23 (SD) % COHb). Suicides also presented higher COHb levels. Older individuals and those with thermal lesions presented lower levels. Politrauma was the most frequent cause of death among the negative cases (24.4%).

It is possible to conclude that forensic aspects of CO poisoning interact in a complex way and differential diagnosis is not straightforward. This study also emphasizes the role played by public prevention campaigns and improvement of heating appliances in reducing the number of unintentional CO poisonings and the importance of preventing urban and forest fires, the major source of CO among us.

KEYWORDS: Carbon monoxide poisoning; Forensic practice; Retrospective study

RESUMO

As intoxicações por monóxido de carbono (CO) são causa de morte frequente e um problema de saúde pública. Analisámos a epidemiologia e aspetos forenses *postmortem* em casos com pedido de análise de carboxihemoglobina (COHb), através do estudo dos relatórios de todas as autópsias realizadas no Serviço de Patologia Forense da Delegação do Centro do Instituto Nacional de Medicina Legal, entre 2000 e 2010. Foram avaliados os seguintes critérios: ano, mês, estação do ano, idade, sexo, profissão, comorbilidade, etiologia, fonte de CO, causa de morte, achados de autópsia e resultados de análises toxicológicas. São objeto de discussão, a importância da informação circunstancial e dos achados de autópsia, a evolução da intoxicação por CO enquanto causa de morte e os desafios do seu diagnóstico diferencial na nossa prática forense.

Entre Janeiro de 2000 e Dezembro de 2010, foram requisitadas 69 análises de carboxihemoglobina na nossa instituição. Em aproximadamente 70% dos casos, a informação circunstancial incluía referência a uma fonte CO identificada no local da morte. Mais de metade dos casos apresentavam lesões térmicas; o livor carminado e a coloração carminada do sangue e vísceras foram identificados em 30%. Neste período, 14 casos (20,3% de todos os pedidos) foram classificados como intoxicações por CO. O maior número de casos verificou-se no ano 2000 e, na restante década, a anos com números superiores de casos, seguiram-se anos sem quaisquer casos. A maior parte das intoxicações ocorreu no inverno (53,8%) e foram mais frequentes em homens (69,2%) e no grupo etário de 51-60 anos (3 casos). 69,2% das intoxicações (9 casos) foram acidentais e as restantes, suicídios. A fonte de CO mais frequentemente identificada foi um incêndio (38,5%). O livor carminado foi identificado em 61,5% dos casos e todas as intoxicações apresentavam coloração carminada do sangue e vísceras, relacionada com concentrações superiores de COHb (58 ± 23 (DP) %COHb).

Também o suicídio se relaciona com concentrações superiores de COHb. Os idosos e os indivíduos com lesões térmicas apresentavam concentrações inferiores. Nos casos com análise negativa, a cause de morte mais frequente foi politrauma (24,4%).

É possível concluir que os aspetos forenses da intoxicação por CO interagem de forma complexa e que o seu diagnóstico diferencial não está desprovido de desafios. Os autores dão ênfase ao papel das campanhas de prevenção junto da população e da evolução dos equipamentos de aquecimento, na redução do número de casos acidentais de intoxicação por CO e à importância da prevenção de incêndios florestais e urbanos, a principal fonte de CO entre nós.

PALAVRAS-CHAVE: Intoxicações por monóxido de carbono; prática forense; estudo retrospetivo

1. Introduction

Carbon monoxide (CO) is a product of the incomplete combustion of organic materials. Natural sources of CO include forest fires and volcanic eruptions but it is mainly produced by human activity [1]. Automobile exhaust fumes, charcoal briquets in confined spaces and defective or improperly ventilated gas heating appliances are common sources of CO. Even though nontoxic natural gas is now the primary source of domestic energy, its combustion with an insufficient supply of oxygen generates CO, with its own deleterious effects [2]. CO also results from heme catabolism and has recently been recognized as a neurotransmitter [3]. Endogenous production and environmental exposure to CO account for baseline levels of carboxihemoglobin (COHb) of less than 1 to 3% in nonsmokers, while smokers may present basal levels up to 10% [1, 4].

CO is odorless, colorless and tasteless. It is not detected by an exposed person and because it has approximately the density of air, it easily spreads in confined spaces [2]. The most generally accepted toxicity mechanism of CO depends on its exceptional affinity for hemoglobin, 200 times greater than that of oxygen, resulting in tissue hypoxia. Today it is recognized that CO may have a direct cellular toxicity [1, 4]. Depending on the concentration of the gas in air, length of exposure and health condition, CO effects range from mild cardiovascular and neurobehavioral symptoms at COHb levels of less than 15 to 20%, to unconsciousness and death. Children, patients with cardiovascular and lung disease and the elderly, are particularly susceptible to CO [4, 5, 6].

In forensic practice, CO-related deaths result mainly from accident or suicide, being homicide rare. Risser and Schneider (1995) report that in 417 CO-related deaths over a ten year study in Vienna, Austria, the majority of cases were accidents, peaking in winter months [6]. These findings are similar to those of Homer *et al.* (2005) in Cleveland, USA and Ait El

Cadi *et al*. (2009) in Morocco [7, 8]. Scene investigation often provides circumstantial indications of CO poisoning and autopsy usually follows. Characteristic pathological findings include cherry-red or bright pink livor and bright cherry-red coloration of blood, musculature and viscera. According to forensic medicine literature, these changes are consistent with levels of COHb greater than 30% [9, 10]. They are important forensic clues to the cause of death, which is established after determination of COHb levels greater than 50 to 60% in *postmortem* blood samples. However, establishing the role of CO as the cause of death is not easy and the interpretation of lower or negative COHb levels in victims with concomitant morbidity may be difficult, namely when excluding natural death and delayed CO-related death cases with suggestive circumstances may present with negligible COHb in blood [11, 12]. On the other hand, cherry-red livor may be absent [13] or result from body refrigeration and cyanide poisoning [12, 14].

CO poisoning has recently been drawing more attention in the popular media. Public awareness of the dangers of CO poisoning, along with improved safety of heating and cooking appliances and gas emission controls in transportation may have been responsible for the decline in the number of unintentional CO-related deaths. Nonetheless, CO poisoning remains an important public health problem and a common cause of death in industrialized countries, being involved in over one half of all fatal poisonings worldwide, yearly [4, 5].

The purpose of this study was to investigate the epidemiology and forensic aspects related to CO poisoning in cases with *postmortem* COHb analysis, from autopsies performed at the Forensic Pathology Department of the Centre Branch of the National Institute of Legal Medicine (NILM) of Portugal between 2000 and 2010.

2. Material studied

Autopsy reports of all deaths examined at the Forensic Pathology Department of Coimbra's Delegation of the NILM of Portugal between January 2000 and December 2010 were reviewed. Actual data material consisted of 69 autopsy reports in which there was a reference to a *postmortem* carboxihemoglobin (COHb) analysis request. COHb analyses were performed at the Forensic Toxicology Department of the Centre Branch of the NILM of Portugal by a molecular absorption spectroscopy methodology [15], in *postmortem* blood samples collected by forensic pathologists. Assessment of the examination protocols was conducted by means of a tabular database according to the following criteria: year, month, season of the year (January, February and March were considered winter months; April, May and June, spring months; July, August and September, summer months and October, November and December, autumn months), age, gender, occupation, underlying disease, medico-legal etiology, source of CO, cause of death, autopsy findings (lividity, thermal injuries, coloration of blood and viscera) and toxicological analysis results. These variables were studied and relevant data was submitted to numerical analysis, using SPSS (Statistical Package for Social Sciences).

3. Results

3.1 COHb analysis requests

3.1.1 Year, month and season distribution

Between January 2000 and December 2010, 69 *postmortem* COHb toxicological analysis were requested and performed at the Centre Branch of the NILM. As presented in Fig. 1, the number of COHb analyses requests was highest in 2005, with 15 cases (21.7% of all requests), number very close to the one achieved in 2000, with 14 cases (20.3%). The years 2002 and 2006 presented the lowest number of requests, 2 (2.9%). The month with the highest number of COHb analysis requests was March (13 cases, corresponding to 18.8%) and winter was the season with the highest number of requests (24 individuals, 34.8% of all requests) (Fig. 1).

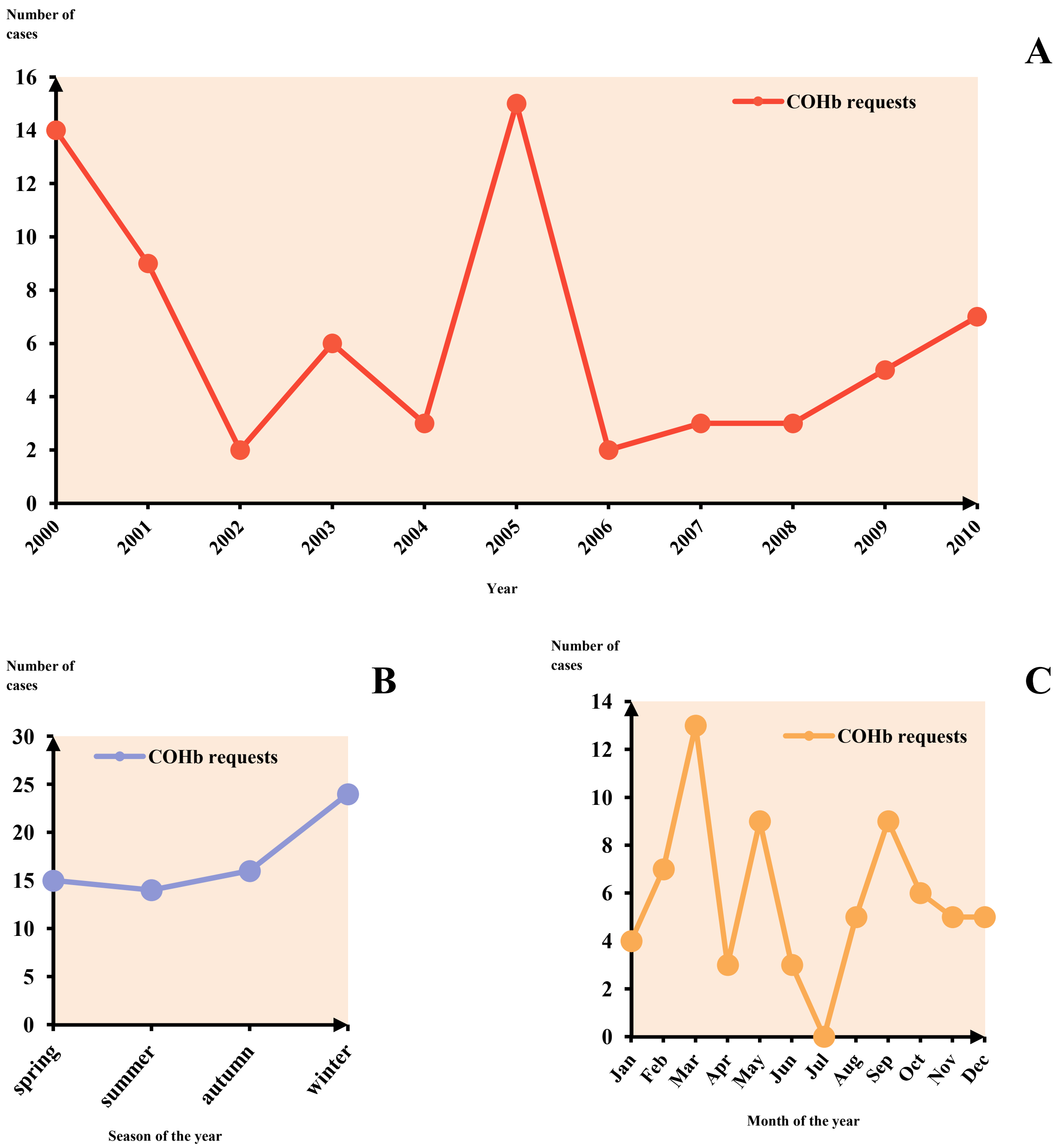

Fig. 1. COHb analysis requests distribution by year (A), season (B) and month (C).

3.1.2 Gender, age and occupation

Distribution of the cases with COHb analysis according to gender and age is presented in Fig. 2. Male individuals represented 63.8 % (44 individuals) of all cases with a COHb analysis request and in 25 female individuals, COHb analysis was requested (corresponding to 36.3% of all individuals with a COHb analysis). Mean age was 55 ± 22 (SD) years. Age group of >70 years had the highest number of COHb analysis requests (22 cases, 31.9%). The age groups of 11-20 years old and <10 years old were the groups with the lowest number of requests (with 1 request - 1.4% - and 2 requests - 2.9%, respectively). In all age groups, except for the two groups with the lowest number of requests, there were more male than female individuals (Fig. 2).

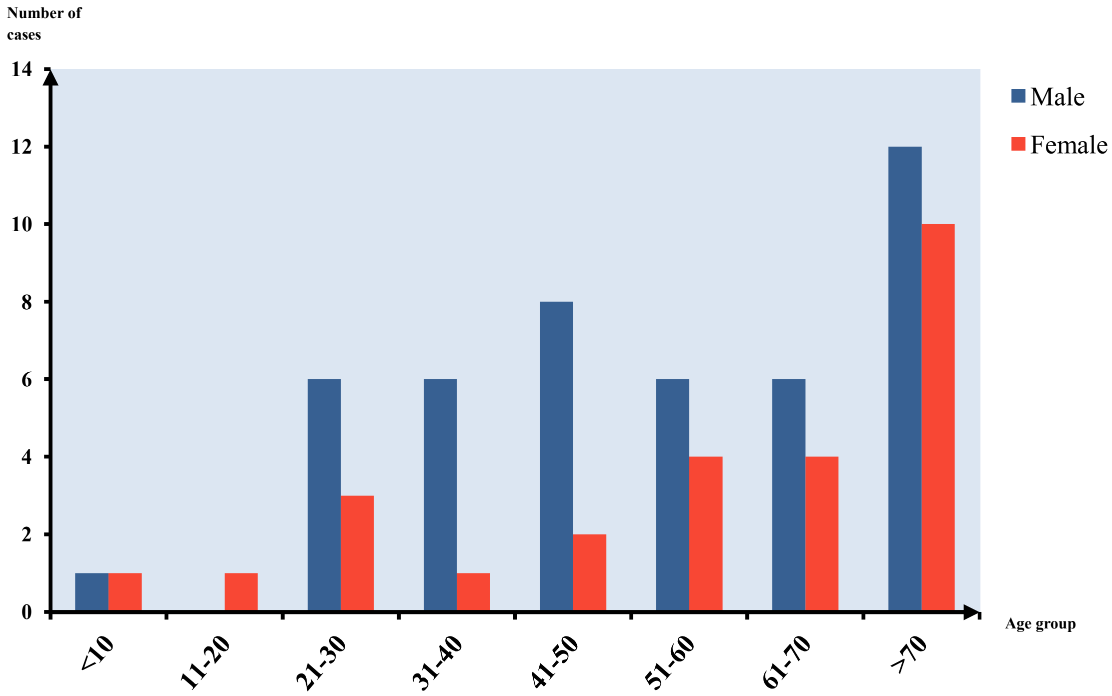

Fig. 2. Distribution of the cases with a COHb analysis request according to gender and age group.

Distribution of the cases with a *postmortem* COHb analysis request by occupation is presented in Fig. 3. In 75.4% of the cases (52 cases), the information about the occupation of the deceased wasn't, *per se*, justification for a CO poisoning suspicion, whether because it was unknown (50.7% of all requests) or because the occupation wasn't associated with an increased risk of CO poisoning (24.6%). In 4 (5.8%) cases, the individual was a fireman and in 13 (18.8%), a pensioner (Fig. 3).

Among the cases with a COHb analysis request that weren't associated with an identifiable source of CO, 4 (19%) individuals were pensioners and in 17 (81%), the occupation was unknown or not associated with an increased risk of CO poisoning.

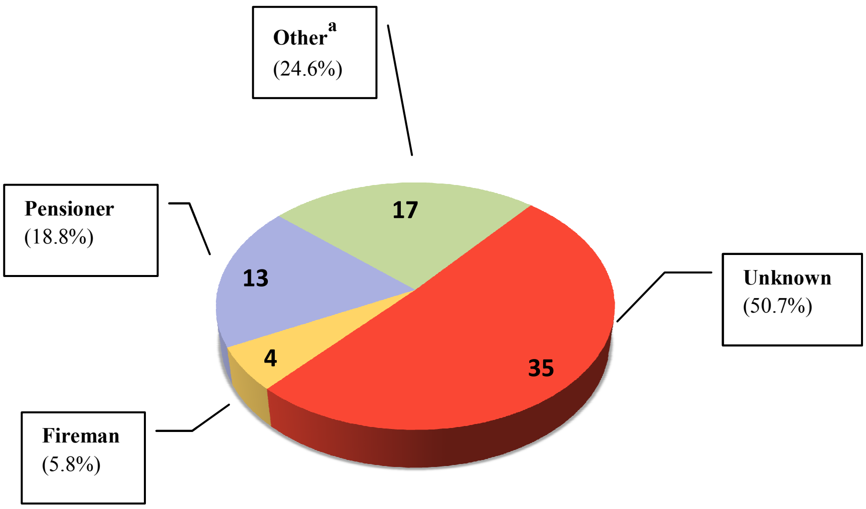

Fig. 3. Distribution of the cases with a COHb analysis request by occupation.

[a] Includes all occupations without an increased risk of CO poisoning and that wouldn't, *per se*, justify an analysis request.

3.1.3 Etiology and source of CO

Characterization of the cases with *postmortem* COHb analysis according to the presence of a source of CO at the death scene and corresponding etiology is described in Table I. Circumstantial information in the autopsy reports indicated that the medico-legal etiology of the death was of natural death in 11 individuals (15.9% of all cases) and in 58 (84.1%) death had a violent etiology. Among the later, 50 individuals (72.5% of all requests) presented circumstantial information that suggested an unintentional death and 6 cases (8.7%) were suicides. Only 2 of the cases (2.9%) were related to homicides (Table I). There was an identified source of CO at the death scene of 48 (69.6%) individuals with a COHb analysis request. 36 individuals (75% of the cases with an identified CO source and 52.2% of all cases with a COHb analysis request) were fire victims.

Table I. Characterization of the cases with COHb analysis request according to the source of CO found at the death scene and corresponding medico-legal etiology.

Source of CO	Analysis requests	Etiology			
		Natural death, *n* (%)	Violent, unintentional, *n* (%)	Suicide, *n* (%)	Homicide, *n* (%)
Fire	36	2 (5.6)	33 (91.7)	0	1 (2.8)
Charcoal briquettes	2	0	1 (50)	1 (50)	0
Burnt wood can	1	0	0	1 (100)	0
Fire pan	1	0	0	1 (100	0
Defective gas appliance	5	0	3 (60)	2 (40)	0
Fireplace	3	0	3 (100)	0	0
Not present	21	9 (42.9)	10 (47.6)	1(4.8)	1 (4.8)
Total	**69**	**11 (15.9)**	**50 (72.5)**	**6 (8.7)**	**2 (2.9)**

Among the deaths classified as suicides, 5 (83.3% of all suicides with a COHb analysis request) individuals presented an identifiable source of CO. One of the two homicides with a COHb analysis request was a fire victim and the other case had no identifiable source of CO at the death scene, but the individual presented thermal lesions.

3.1.4 Autopsy findings

Relevant autopsy findings of the cases with a *postmortem* COHb analysis request between January 2000 and December 2010 are presented in Table II. 30.4% (21 cases) of the individuals presented cherry-red lividity. In 29% of the cases, corresponding to 20 individuals, lividity was *bluish* and in 40.6% (28 individuals), lividity wasn't characterized because of skin pigmentation or large extent of thermal lesions. Among the cases in which a source of CO was identified at the death scene, in the highest number of cases (54.2%, corresponding to 26 individuals), lividity couldn't be characterized; on the other hand, in this group, cherry-red lividity was more frequent than *bluish* lividity (31.3% - 15 cases - vs. 14.6% - 7 cases). Moreover, in the group of requests without an identifiable CO source, *bluish* livor was more frequent (61.9%, corresponding to 13 cases) but in 6 (28.6%) cases, lividity was cherry-red. Thermal lesions were described in 56.5% (39 cases) of all cases with a *postmortem* COHb analysis request. Cherry-red coloration of the blood and viscera was found in 21 cases, corresponding to 30.4% of the individuals with a COHb analysis request. This finding was described in 41.7% (20 individuals) of the cases with an identified source of CO. (Table II). In 7 cases (10.1% of all cases with a COHb analysis request), no source of CO, poisoning associated to occupation or suggestive autopsy findings were described. All these cases had a negative *postmortem* COHb analysis.

Table II. Characterization of the cases with COHb analysis request according to the source of CO found at the death scene and corresponding autopsy findings.

Source of CO	Analysis requests	Autopsy findings					
		Lividity			Thermal lesions *n* (%)	Cherry-red viscera *n* (%)	Soot *n* (%)
		Cherry-red, *n* (%)	*Bluish*, *n* (%)	Not characterized [b], *n* (%)			
Identified [a]	48	15 (31.3)	7 (14.6)	26 (54.2)	35 (72.9)	20 (41.7)	25 (52.1)
Not present	21	6 (28.6)	13 (61.9)	2 (9.5)	4 (19)	1 (4.8)	0
Total	**69**	**21 (30.4)**	**20 (29)**	**28 (40.6)**	**39 (56.5)**	**21 (30.4)**	**25 (36.2)**

[a] Includes fire, charcoal briquettes, burnt wood can, fire pan, defective gas appliance and fireplace.

[b] Refers to the cases in which the lividity was not characteristic, because of skin pigmentation or large extent of thermal lesions.

3.2 Positive analyses

3.2.1 COHb levels according to gender and age

Between January 2000 and December 2010, 24 individuals (34.8% of all cases with a *postmortem* COHb analysis request) had a positive COHb analysis. We verified that in these cases, the lower COHb levels were achieved in the older people (Pearson Correlation Coefficient). Mean % COHb in positive cases was similar in male and female individuals (49 ± 27 vs. 51 ± 16 (SD) % COHb; Student's *t*-Test for Independent Samples, p=0.952).

3.2.2 COHb levels according to autopsy findings

Out of 24 individuals with a positive analysis, 9 cases (corresponding to 37.5%) presented cherry-red lividity (in 2 cases - 8.3%, lividity was *bluish* and in 13 cases (54.2%), lividity wasn't characterized). We found that in individuals with COHb and characterized lividity, the type of lividity was not dependent on the COHb percentage (Mann-Whitney U

Test for Independent Samples, *p*=0.906). 15 cases (62.5% of all positive requests) presented cherry-red coloration of blood and viscera. We verified that positive cases with cherry-red coloration of blood and viscera had significantly higher COHb levels than the positive cases in which this finding was absent (58 ± 23 (SD) vs. 32 ± 17 (SD) % COHb; Mann-Whitney U Test for Independent Samples, *p*=0.012). The lowest COHb level in a positive case that presented cherry-red lividity was 3% COHb, which is similar to the lowest COHb level in a positive case that presented cherry-red blood and viscera coloration. The highest level of COHb in a positive case with *bluish* lividity was 82%. The highest level of COHb in a positive case without cherry-red blood and viscera coloration was 61%. Among the 13 cases with a positive analysis in which lividity wasn't characterized, 6 individuals (46.2%) presented cherry-red blood and viscera coloration. 15 individuals (65.5%) with a positive analysis also presented thermal lesions. In this group, mean % COHb was significantly lower than that of the group of individuals with a positive analysis, without thermal lesions (41 ± 24 vs. 64 ± 18 (SD) % COHb, Mann-Whitney test, *p*=0.025).

3.2.3 COHb levels according to cause of death

Among the positive cases, cause of death was not CO poisoning in 11 cases (45.8% of the positive cases). In this group, 9 individuals (81.8% of the positive cases who did not die due to CO poisoning and 37.5% of all the positive cases) died because of carbonization and 2 individuals (8.3%) because of burns. Mean COHb level in individuals with a positive analysis whose cause of death was not CO poisoning, was of 32 ± 19 (SD) % COHb. The highest COHb level in a positive case whose death was not caused by CO poisoning was 61% COHb. In the group of cases with a positive analysis in a death not caused by CO poisoning, cherry-red lividity was found in only 1 individual (9.1%); 1 individual (9.1%) presented with *bluish* lividity and, in the majority of the cases (9 individuals, 81.8%), lividity couldn't be characterized because of the extension of thermal lesions. Also in the group of cases with a positive analysis in a death not caused by CO poisoning, cherry-red blood and viscera coloration was found in 3 individuals (27% of the cases); among the cases with a positive analysis and a cause of death different of CO poisoning in which lividity couldn't be characterized because of thermal lesions, 2 individuals (22.2% of these cases) presented cherry-red blood and viscera coloration.

3.2.4 CO poisonings

3.2.4.1 Year, month and season distribution

CO poisoning was the cause of death in 14 cases with a *postmortem* COHb analysis request between January 2000 and December 2010, representing 20.3% of all cases with an analysis request. This group included 13 cases (92.9% of all CO poisonings) with a positive analysis and 1 case (7.1%) in which the *postmortem* COHb analysis was negative.

Yearly distribution of CO poisonings with a positive analysis is presented in Table III. 2000 was the year with the highest number of fatal CO poisonings with positive COHb analysis (5 cases, 38.8% of all positive CO poisonings). The highest number of positive, fatal CO poisonings, occurred in February, March and May (3 cases in each month, corresponding to 23% of all poisonings with a positive analysis). The remainder occurred, equally, in January, February, April, November and December (1 case in each month, corresponding to 7.7% of the positive poisonings). Winter was the season with the highest number of these cases (7 cases, corresponding to 53.8% of all positive poisonings). 4 cases (30.8%) occurred during spring and 2 (15.8%) during autumn. No cases occurred during summer.

3.2.4.2 Gender, age and occupation

As presented in Table III, male individuals account for the great majority of the cases related to CO poisonings with a positive analysis, with 9 individuals (69.2%). Mean age in the group of positive fatal CO poisonings was 42 ± 26 (SD) years. Distribution of positive fatal CO poisonings by age groups is presented in Fig. 4. In 61.5% (8 individuals) of positive CO poisonings, the victim's occupation was unknown. In 2 cases (15.4%), the individual was a pensioner. Student, plumber and waitress accounted for the remaining 3 cases (1 case each).

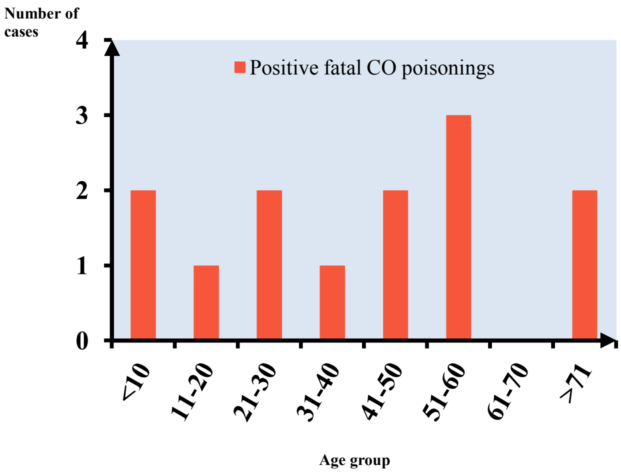

Fig. 4. Distribution of the fatal CO poisonings with positive COHb analysis, by age group.

Table III. Yearly distribution of the CO poisonings with positive COHb analysis and corresponding characterization according to gender and etiology of death.

Year	Positive CO poisonings	Gender		Etiology	
		Male, *n* (%)	Female, *n*(%)	Violent, unintentional, *n* (%)	Suicide, *n* (%)
2000	5	4 (80)	1 (20)	5 (100)	0
2001	3	1 (33.3)	2 (66.7)	2 (66.7)	1 (33.3)
2003	1	1 (100)	0	0	1
2008	3	3 (100)	0	1 (33.3)	2 (66.7)
2010	1	0	1(100)	1 (100)	0
Total	**13**	**9 (69.2)**	**4 (30.8)**	**9 (69.2)**	**4 (30.8)**

3.2.4.3 Etiology and source of CO

69.2% (9 cases) of the positive CO poisonings were violent, unintentional deaths. The remainders were suicides (4 cases, corresponding to 30.8% of positive CO poisonings). Considering our population, CO poisoning was the cause of death in all 4 suicides that presented with positive levels of COHb in a *postmortem* blood sample. The most common CO source among the positive CO poisonings were the fires (5 cases, corresponding to 38.5%). In 3 cases (23.1%), a defective gas appliance was found at the death scene. In 2 cases (15.4%), the sources of CO were burning charcoal briquettes in a confined space. A fireplace (1 case, 7.7%), a can containing burnt wood (1 case, 7.7%) and a fire pan (1 case, 7.7%) were also found among these cases. Source of CO was different in all 4 CO poisoning suicides, having been identified 1 defective gas equipment, 1 can containing burnt wood, 1 fire pan and, in 1 case, charcoal briquettes.

3.2.4.4 Autopsy findings

Cherry-red lividity was present in 8 of 13 (61.5%) positive fatal CO poisonings; in 4 (30.8%), lividity wasn't characterized because of skin pigmentation or thermal lesions; in 1 (7.7%) case, lividity was *bluish*. Nonetheless, all the 13 positive fatal CO poisonings presented cherry-red blood and viscera coloration.

3.2.4.5 Toxicological results

Mean % COHb in the cases of individuals with positive COHb analysis who died because of CO poisoning was 65 ± 18 (SD) %. Older individuals with a positive fatal CO poisoning presented lower levels of COHb % (Pearson Correlation Coefficient). Also, COHb levels were significantly higher among positive fatal CO poisonings of suicidal etiology than in violent, unintentional, positive fatal CO poisonings (78 ± 17 vs. 58 ± 15 (SD) % COHb;

Kruskal-Wallis Test for Independent Samples , p=0.013). The lowest COHb level found in a CO poisoning with a positive analysis was 42% and 4 (30.8% of the cases) out of the 13 individuals who died due to CO poisoning which presented COHb levels below 50%. Medical history was unknown in these 4 cases and only 1 (25%) presented thermal lesions. None of these 4 individuals was alcohol intoxicated. Analysis for illicit drugs was negative in 1 of these cases (25%) and wasn't performed in 3 cases (75%). The same results were verified for prescription drugs analysis. Moreover, we found that all the 11 positive CO poisonings in which blood alcohol was tested (84.6% of all positive CO poisonings), were not alcohol intoxicated. Illicit drugs were analyzed in 5 cases (38.5%), all with negative results. In all 4 cases with a prescription drug analysis (30.8%), results were negative.

3.3 Negative analyses

Between January 2000 and December 2010, 45 individuals (65.2% of all requests) had a negative *postmortem* COHb analysis. This group included 26 males (57.8%) and 19 females (42.2%).The mean age among these individuals was 60 ± 19 (SD) years. Medico-legal etiology was of natural death in 10 cases (22.2%); violent, unintentional death in 31 cases (68.9%); suicide in 2 cases (4.4%) and homicide in 2 cases (4.4%). Politrauma was the most frequent cause of death among the negative cases (11 cases, 24.4%). Among natural causes of death, acute myocardial infarction was the most frequent, with 4 cases (8.8% of all negatives). In 1 case (2.2%) of an individual with a negative analysis request, CO poisoning was the determined cause of death. This former case, an accident involving a fire, presented cherry-red blood and viscera coloration, but *bluish* lividity and no thermal lesions.

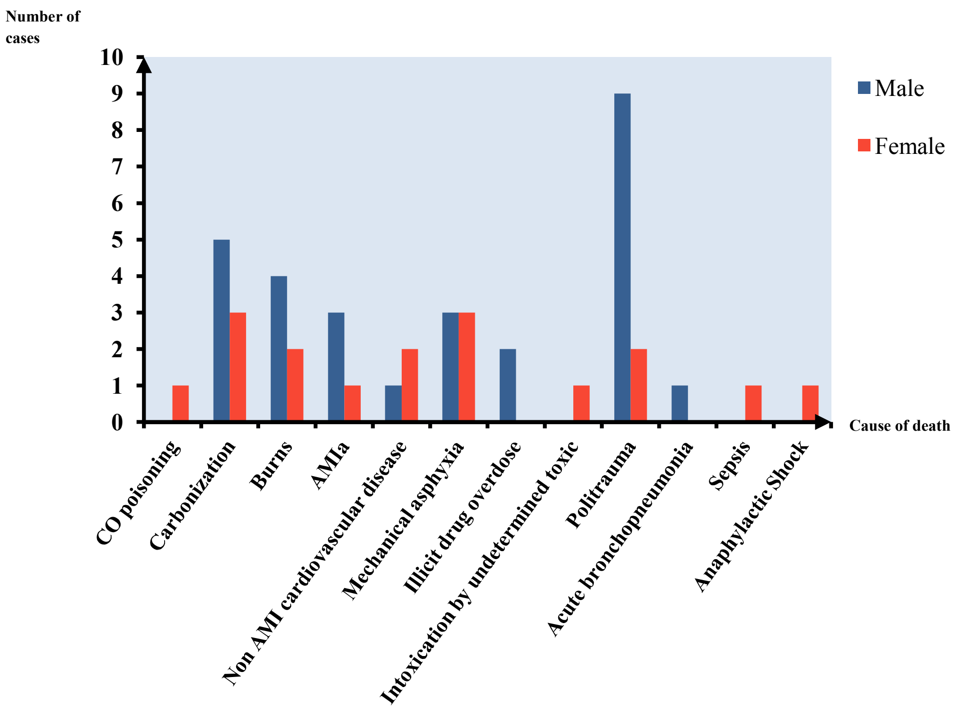

Fig. 5. Cause of death in males and females with a negative COHb analysis.

[a] *AMI*, acute myocardial infarction.

4. Discussion and conclusion

In Portugal, incidence of CO poisoning is 5.86/100000, representing 0.04 and 0.05% of internal medicine admissions in rural and urban areas, respectively [16]. A surveys of fatal cases registered after the fifties was carried out in our country [17] but it also studied the remaining intoxications, thus presenting few conclusions on the characterization of the CO-related deaths analyzed. Moreover, in international publications, studies of this nature reviewed only deaths in which CO poisoning was the cause of death, thus leaving uncharacterized the cases in which there was an unconfirmed suspicion of CO poisoning. Frequently, the population was a specific group of fatal CO-related deaths, selected according to etiology and source of CO [6, 7, 18, 19, 20]. Also, characteristic autopsy findings and their relevance in the differential diagnosis of CO poisoning as a cause of death have only rarely been studied in surveys of fatal CO poisonings [10]. Instead, this subject has been addressed in case reports of peculiar CO-related deaths [11, 13, 21] or publications of other natures [12].

By including in our study, not only the deaths caused by CO poisoning, but also those in which the suspicion of CO poisoning was not confirmed (cases with a positive analysis but a different cause of death and cases with a negative analysis), we analyzed the problem of CO poisoning as a cause of death and differential diagnosis in the forensic practice through a broader perspective than that of studies of this nature previously made. The lack of national publications regarding the characterization of fatal CO poisonings and the fact that our study design is unparalleled by similar international publications make our results difficult to discuss taking all variables studied into account but some aspects in these study allow us to point out trends in fatal CO poisoning over the last 11 years and also present, in one hand, a number of circumstantial and autopsy findings that lead us, in our practice, to a CO poisoning suspicion and subsequent COHb analysis request and, on the other hand, the major differential diagnosis of this type of intoxication, among the analyzed cases.

We observed that the number of COHb analysis requests peaked in 2000 and 2005 which have been, interestingly, the years with the highest number of forest fires in Portugal, during the decade. In our study, the source of CO most frequently found at the death scene was indeed a fire but the highest number of requests was made in March and, seasonally, during winter, when forest fires are less frequent. Thus, our yearly, seasonal and monthly distribution of COHb analysis requests is probably more directly related to the prevalence of urban fires rather than that of forest fires. Our results are in accordance with the generalized idea that CO poisoning is more frequent during winter months, because of the increased use of heating appliances and diminished house ventilation [22], confirmed according to our national reality by Sá *et al.* (2011) [16]. The above considerations also explain the seasonal distribution of fatal CO poisonings which were, in our study, more frequent during winter months and non-existent during summer, results paralleled by those of other authors [6, 7, 8, 19, 23]. The yearly distribution of COHb requests was not paralleled by the distribution of fatal CO poisoning cases, which were more prevalent in 2000 and never reached so high numbers through the remaining of the decade. Pinho Marques *et al*. (2002) reported 42 fatal CO poisonings between 1990 and 1999, the decade with the highest number of cases since the fifties, following the overall increase in the number of all intoxications registered [17]. The comparative decrease in the number of fatal CO poisonings between 2000 and 2010 was expected, due to improved treatment (namely the hyperbaric chamber in Matosinhos) [16], the opening of new medico-legal facilities and the impact of these cases in national public media and prevention campaigns directed to the population, which could also explain that years with more CO poisonings were followed by years with no CO poisonings (Table III).

Other authors reported that fatal CO poisonings are more prevalent among males [7, 18, 24], an observation also supported by our own results (Table III). It has been suggested by Nazari et al that men engage in high-risk behaviors such as manual work with fuel-burning tools or appliances and their maintenance, more often than women [23]. In our study, fire was the most common source of CO (Table I) and, according to Blanc and Kuschner (2007), more than one third of all firemen use protection masks less than half the time, during a fire suppression [25]. Moreover, in Portugal, firemen still are mainly male individuals. However, occupation was unknown in the majority of CO poisonings, which makes it difficult to understand the role of occupation in gender differences.

The age group with the highest number of cases was between 51 and 60 years-old (Fig. 4), while most authors reported an higher prevalence among the youngest and the eldest [7, 18]. These studies considered unintentional CO-related deaths only and therefore, this difference may be explained by the particular age distribution of CO-related suicides, described by Risser and Schneider (1995) [6]. Moreover, we found that other age groups, namely those of children (<10), the young (21-30), middle aged (41-50) and the eldest (>70) are also at risk (Fig. 4). The young, despite having higher body resistance, are less careful and preventive [19] and the elderly are at risk because of their limited mobility, poorer economic situation, likelihood of mistaking CO poisoning for fatigue or influenza-like illness, and comorbility [23]. Children are more susceptible to CO poisoning because they have an higher metabolic rate and oxygen uptake [4].

The majority of requests for a COHb analysis were made for deaths of violent, unintentional nature (Table I) and, not unexpectedly, we verified that CO poisonings were mainly unintentional (Table III), which is in accordance to the literature [6, 8, 19]. Interestingly, in a five year study in East Denmark, Theilade (1990) reported an higher relative number of suicides, which is possibly related to the high incidence of suicide in countries of northern Europe [24]. In our study, a request was made in 6 suicides, representing 8.7% of all requests (Table I), an expected percentage considering that Sá *et al.* (2011) found that, in seven hospitals of northern Portugal, the prevalence of CO-related intoxications resulting from attempted suicide was 10.8% [16]. On the other hand, our percentage of CO-related suicides was 30.8% (Table III). CO intoxications of suicidal nature are more frequently fatal because, as verified in our study and by Risser and Schneider (1995), their COHb levels are usually higher [6] and thereby it is not surprising that the prevalence of suicidal cases is higher when considering fatal cases only. However, our percentage of suicides is also higher than that of other studies of fatal cases [6, 8, 19]. We believe that may be due to the efficiency of prevention measures in reducing the number of unintentional deaths, rather than to an absolute increase in CO-related suicides.

In our study, the source of CO was a heating appliance in all CO-related suicides. Unlike other studies [6, 24], we registered no suicides by inhalation of car exhaust fumes. Studies such as the one of Risser and Schneider (1995), in which this was the most frequent suicide method, refer to the late eighties and early nineties, when catalytic converters were still in development [6]. Most unintentional CO poisonings resulted from fires, as other authors also reported [7, 24]. This result emphasizes, first, the efficiency of safety improvements in heating appliances and public awareness and, secondly, the necessity to further invest in fire prevention, namely through the mass generalization of smoke detector installation in Portuguese homes. In fact, some authors have reported that a working smoke detector reduces the risk of death by fire in the home at least 50% [7]. We found that in most requests, information that a CO source had been identified at the death scene was available and out of these cases, most did not present with characteristic autopsy findings that could suggest the hypothesis of CO poisoning, to the medico-legal expert (Table II), thus confirming the presence of a CO source in the death scene as a putative motivation to request a COHb analysis and the overall importance of circumstantial information in forensic toxicology, as debated by Reys and Santos (1992) [26].

These authors also reported autopsy findings to be a decisive factor for a toxicological analysis request in an important number of cases [26]. Accordingly, we verified that nearly one third of the cases with a COHb analysis request presented with characteristic cherry-red lividity and the same percentage of cases presented cherry-red blood and viscera coloration. Also, these findings were present in some cases in which a source of CO was not identified. In over one half of the cases, the victims presented thermal lesions (Table II). These results allow us to conclude that, in our practice, the presence of a source of CO at the death scene and suggestive autopsy findings concur to a strong suspicion of CO poisoning. The absence of cherry-red lividity is not, however, conclusive evidence that CO poisoning was not the cause of death. In fact, cherry-red livor was present in only 61,5% of CO poisonings, a percentage much lower than that of Risser et al (1995) [10]. Also unlike the former authors, we found no association between COHb levels and the lividity type. A possible explanation could be that we also considered CO-poisonings caused by fire and therefore, our cases included those in which lividity was not characterizable due to external thermal lesions but that could, otherwise, have presented cherry-red lividity. This is supported by the fact that in our study, cherry-red blood and viscera coloration, not influenced by the above factors, was present in all CO poisonings, including those without cherry-red lividity, and had a strong association with higher COHb levels. On the other hand, we found that cases with a positive analysis and thermal lesions were strongly associated with lower COHb levels, as also reported in other studies [20, 24, 27]. It is known that burns, instead of CO poisoning, is the cause of death in at least thirty percent of fire victims [27]. However, fire victims that were alive when the fire started will likely present positive COHb levels and even in nonfatal concentrations, CO may contribute to death. In fact, death by fire frequently results of a combinations of these causes [20]. Among the CO poisonings in our study, we observed a mean % COHb of 65%, which is in conformity with the widely accepted threshold for fatal cases, 50 or 60% [9]. Older individuals presented lower COHb levels and this was also reported by Risser and Schneider (1995) [6]. It is known that the elderly with underlying cardiovascular disease are less tolerant to CO pathogenicity [6, 20] and this could explain our 4 cases in which CO poisoning was the cause of death but presented with COHb levels under 50%. Disappointingly, no medical history was available in all cases.

Blood alcohol, prescription and illicit drug analyses were negative in all CO poisoning cases in which they were performed. These substances have been related to an increased risk of CO-related accidents, because they impair the decision making capability, and to individuals who ingest large quantities of drugs before committing suicide through CO exposure [20, 28]. On the other hand, Przepyszny and Jenkins (2007), found that the presence of drugs did not change the cause of death in any of their cases [28].

It is not surprising that politrauma was the most common cause of death among our negative cases (Fig. 5). As previously discussed, circumstantial information is a decisive motivation to request a toxicological analysis and many of the fire victims in our study were involved in car accidents and explosions, situations that resulted in fatal craneoencephalic or toracoabdominal lesions but in which CO could have played an important role as the cause of death. The same applies to deaths due to thermal lesions, also an important differential diagnosis among unintentional deaths (Fig. 5). Differential diagnosis with natural death and particularly with cardiovascular disease is also a reality in our forensic practice (Fig. 5). As discussed before, the elderly, more prone to severe cardiovascular disease, are also a group at risk for CO poisoning and cases in which, due to unspecific circumstancial information, absence of cherry-red livor and presence of arteriosclerosis and coronary arteries calcification, differential diagnosis was not straightforward, have been reported by other authors [13]. The fact that delayed death by CO poisoning is consistent with negligible COHb levels may impose further challenges [21].

In summary, we have demonstrated that, in our practice, forensic aspects of CO poisoning interact in a complex way. Establishing CO poisoning as a cause of death thus requires a strong suspicion, based on suggestive circumstantial information and autopsy findings, and levels of COHb higher than 50%. Even when these factors are reunited, differential diagnosis is not straightforward. This study also emphasizes the role played by public prevention campaigns and improvement of heating appliances in reducing the number of unintentional CO poisonings and the importance of encouraging further prevention measures regarding urban and forest fires, still the major source of CO among us.

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