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Post-mortem toxicology in the diagnosis of sudden death in young and middle-aged victims

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Abstract. – OBJECTIVE: We aimed to investigate the impact of the toxicological results found in cases of sudden death (SD) and to correlate the clinical, autopsy and genetic findings with the toxicology results.

MATERIALS AND METHODS: Consecutive SD in people aged between 16 and 50 years with medico-legal autopsies and toxicology studies were included over a 3-year period. The comparison between the toxicological data and demographic characteristics, clinical circumstances, autopsy, and genetic results were taken into account.

RESULTS: 101 cases were finally included. They were predominately males (84%) and the mean age was 39.8 years. 52 (51.5%) cases had positive toxicological findings and in 25 cases (24.8%), toxic compounds were considered the first cause of death. Ethanol was the most frequently identified agent (69%), following by licit drugs (56%) and drugs of abuse (39%). Cases with positive toxicology were younger than those with negative results (37.9±9.1 *vs.* 41.9±7.8; *p*=0.02). Patients with more than 3 comorbidities showed an association with positive toxicological results (n=14 *vs.* n=3; *p*=0.017). The genetic study was performed in 70 (69.3%) SD cases. We identified pathogenic or likely pathogenic variants in 17.1% cases and uncertain significance variants in 42.8% cases. 58% of these variants were probably related to the cause of death.

CONCLUSIONS: A large fraction of SD victims had positive toxicological findings and a quarter of deaths were directly caused by toxic substances. The identification of the factors that trigger SD provides a good approach to contribute in avoiding future episodes.

Key Words
Sudden death, Sudden cardiac death, Toxicology, Post-mortem, Drugs, Cardiotoxicity.

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Introduction

Sudden death (SD) in the youngsters is always a tragic and devastating event for the family and the community in general, as it occurs in apparently healthy people[1,2]. Persons having an undiagnosed severe illness or an unfavorable genetic disposition may be at risk. In the general population aged from 20 to 75 years, the mean yearly incidence of SD was 1 in 1,000 inhabitants accounting for the 18.5% of all deaths[3].

Nevertheless, the incidence of SD widely varies between different studies. Mainly, this is due to differences in the age range of the study populations. In addition, investigations are often limited by small sample sizes and retrospective and non-population-based study designs. The vast majority of cases are considered to be sudden cardiac death (SCD). In adults, the incidence of SCD increases dramatically with age, in parallel with the age-related increase of coronary heart disease. Also, SCD is substantially lower in women than in men at all ages[1-4]. In Europe, the reported incidence in young people ranged 1.8 to 6.2 per 100,000 per year[5,6]. In Spain, the incidence of SD in the general population is 12% of all deaths, with 88% of these being SCD[7,8]. In the 15-year to 49-year age group, the reported incidence in the north of Spain for SD was 11 per 100,000 person-years[9].

The use of drugs (licit and illicit) and toxic substances, such as ethanol, are a well-known risk factor for SD, frequently associated with cardiovascular origin[4]. However, only a few studies have focused on the role of this association. Two studies carried out in Denmark showed that 47% and 57% of SCD cases were positive for some licit or illicit drug, respectively[4,10].

Some therapeutic drugs, and particularly psychotropic ones, are associated with acquired long-QT syndrome and drug-induced pro-arrhythmia[11]. Other drugs, like anti-arrhythmic drugs, reduce cardiac excitability and can also produce QT interval prolongation and proarrhythmia. Brugada syndrome has also been linked to SCD. There are several drugs, which produce an electrocardiographic pattern typical of Brugada syndrome. Therefore, these drugs are associated with an arrhythmic risk and should be avoided in patients diagnosed with this syndrome[12,13].

The recreational use of illicit drugs remains an enormous and growing problem around the world[14]. Illicit drugs may lead to chronic illnesses requiring therapy, and there is the ever-present risk for sudden catastrophic events[15]. Many illicit drugs, such as cocaine and amphetamines, have been associated with an increased risk of cardiovascular events. These events can be listed as supraventricular and ventricular arrhythmias, acute myocardial infarction**,** and ventricular hypertrophy[15,16]. Moreover, different cases of SD associated with the use of new psychoactive substances, such as synthetic cannabinoids or cathinones, have been documented[17,18].

Autopsy results may help to identify a SD. However, SD can only be diagnosed with certainty if sufficient details on the circumstances surrounding death, the medial history, a full toxicology and microscopic investigation are available. Although autopsy represents the first and last chance for an accurate diagnosis in SD, a significant number of cases in the young remain unexplained after a comprehensive autopsy examination. The Association for European Cardiovascular Pathology has published a protocol for autopsy investigation in SCD. This Association strongly advocates the establishment of regional multidisciplinary specialist networks to improve the diagnosis of SCD[4].

In this concern, the objectives of this study were: 1) to investigate the impact of the toxicological results found in cases of SD in young and middle-aged adults, and 2) to relate the clinical, autopsy and genetic findings with the toxicology results.

Materials and Methods

Study Design

We investigated the reports of SD that occurred in the Balearic Islands between 2015 and 2017. The SD in the Balearic Islands study (MUerte Súbita Islas Baleares, MUSIB) consists of a protocol-based study of all non-traumatic and non-violent SD in individuals between 16-50 years of age occurring in the Balearic Islands (Spain). The study covers an area of 4992 km² and a reference population of 1,115,999 inhabitants according to the 2017 census. Furthermore, the population substantially increases in the summer season, as the Balearic Islands, especially Mallorca and Ibiza, are important tourist destinations. The MUSIB study was performed by a multicentre and multidisciplinary working group formed by cardiologists, forensic experts, pathologists, intensive care specialists, geneticists, molecular biologists**,** and toxicologists. The protocol consists of carrying out a complete medical-legal autopsy in all cases,

with an autopsy, toxicological, and genetic study (guided by histological findings), in an attempt to determine the causal diagnosis of the SD.

The inclusion criteria were: (a) SD in individuals between 16-50 years, (b) no signs of violent death, and (c) available toxicological findings. The MUSIB report includes: clinical characteristics of SD, circumstances of death, prior medical and family history, toxicological findings, macro and microscopic pathological findings, pathogenic mutations resulting from the genetic analysis (if indicated), and probable cause of death. Information on prior medical history was retrieved from both the forensic report and the health history record. This record contains information on all in-patient and out-patient activity in the Balearic Islands hospitals. SD with positive and negative toxicology profiles were compared with regard to clinical characteristics, details concerning the death, comorbidity**,** and cause of death. The study was approved by the Research Ethics Committee of the Balearic Islands.

Forensics

Forensic autopsies were performed according to the guidelines for the autopsy investigation of cardiac SD from the Association for European Cardiovascular Pathology[4]. The named protocol included: clinical antecedents of the case, death scene investigation, complete macroscopic autopsy with the weight and examination of all organs**,** and histological studies of all organs. During the autopsy, blood, vitreous fluid, and urine were collected for toxicological analyses. Enzyme immunoassay, high-performance liquid chromatography and gas chromatography-mass spectrometry were used for the detection of therapeutic drugs, drugs of abuse, and ethanol. The blood levels of each substance were classified as toxic or non-toxic according to previously established therapeutic and toxic blood concentrations[19,20].

In cases that had no cause and had been identified after the protocolized autopsy, 218 cardiac genes were analyzed for a clinically relevant cardiac gene mutation. Genetic analyses were performed using Next Generation Sequencing (NGS)[21]. The study includes the analysis of a panel of genes, which have either previously been associated or considered as candidates, for the development of hereditary cardiovascular diseases. The regions of low coverage of the relevant genes were re-sequenced by the Sanger method[22]. The probable causes of death were discussed in multidisciplinary sessions.

Definitions

Sudden death was defined as a sudden, natural, and unexpected death, in witnessed cases as an acute change in cardiovascular status with time to death being <1 h or, in unwitnessed cases, as a person last seen alive and functioning normally <24 h previously[4].

Depending on the underlying cause, SD can be divided into Sudden Cardiac Death (SCD), defined as SD from a cardiac cause, and SD due to non-cardiac causes[4]. Sudden Unexplained Death (SUD) was defined as an SD where both the autopsy and toxicology investigations remained inconclusive (i.e. non-cardiac causes were excluded, the heart was structurally normal and the positive toxicological findings were determined not to have caused the death)[1]. The cases without mutations suggesting a channelopathy were also included in this group.

The toxic cause of SD was established when the toxicological findings could explain the death. Positive toxicology was defined as the presence of any substance (licit and/or illicit) upon forensic toxicological investigation. The age cut-off for the studied population was defined as 30 years, because SD is 100 times less frequent in these individuals than in adults >35 years[23]. Polypharmacy was defined as the finding of 5 or more concurrent drugs[24].

The comorbidities were grouped into none, one, two, three and more than three. These comorbidities included: psychiatric disease, history of drug abuse, alcoholism, previous heart disease, cardiovascular risk factors (hypertension, smoking status, diabetes mellitus, obesity, and dyslipidemia), asthma, obstructive sleep apnea, and epilepsy.

The variants identified in the genetic study have been classified using a specific standard terminology: pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), or benign (B) (following recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology)[25].

Statistical Analysis

The comparison of the qualitative variables between groups (positive and negative toxicology) was performed using the Chi-square test and the correction of Yates (where appropriate). The comparison of the quantitative variables was performed using the Student's *t*-test. SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for data analysis and statistical evaluation. p-values <0.05 were considered statistically significant.

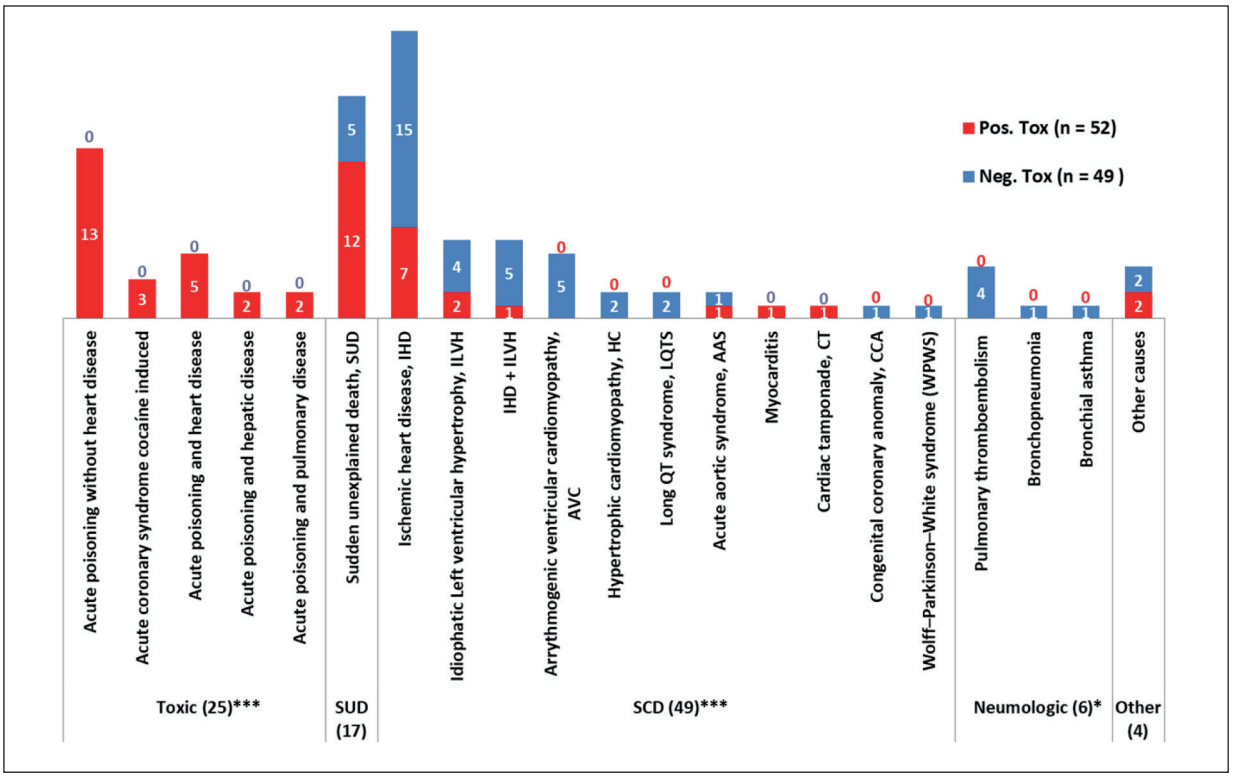

Figure 1. Causes of sudden death. *p<0.05 between toxic and non-toxic groups; ***p<0.001 between toxic and non-toxic groups (Chi-square test).

Results

Impact of the Toxicological Results Found in Cases of SD

In the selected period, 101 SD cases met the inclusion criteria: 88 cases were from Mallorca, 7 were from Menorca, and 6 were from Ibiza/Formentera Islands. In total, 51.5% (n=52) of the cases presented a positive toxicological finding. Toxic substances were considered the first cause or a fundamental cause of death in 25 (24.8%) SD cases (Figure 1). In total, 138 substances (36 different) were detected (Figures 2 and 3). The average number of substances detected in toxicology positive cases was 2.6 (median 2, range 1-6). In 12% (n=12) of cases, polypharmacy was detected. The most frequently

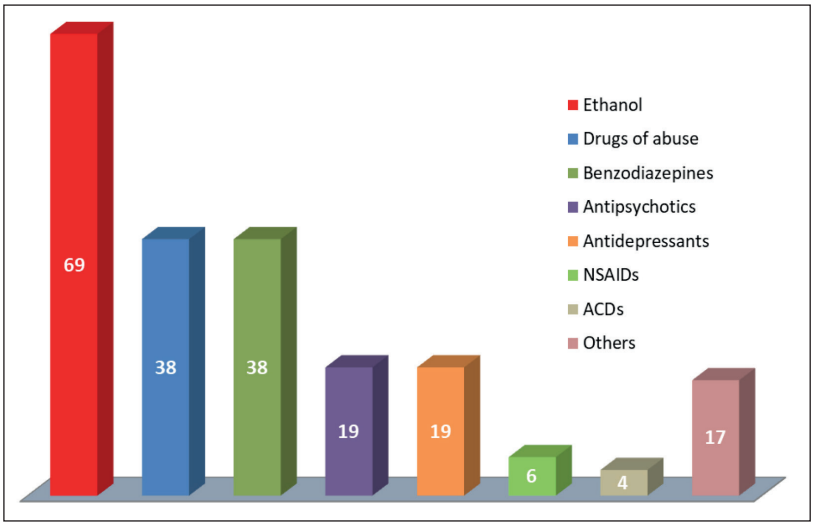

Figure 2. Detected substances in cases with positive toxicology. NSAIDS: Non-steroidal anti-inflammatory drugs; ACDs: Anticonvulsant drugs.

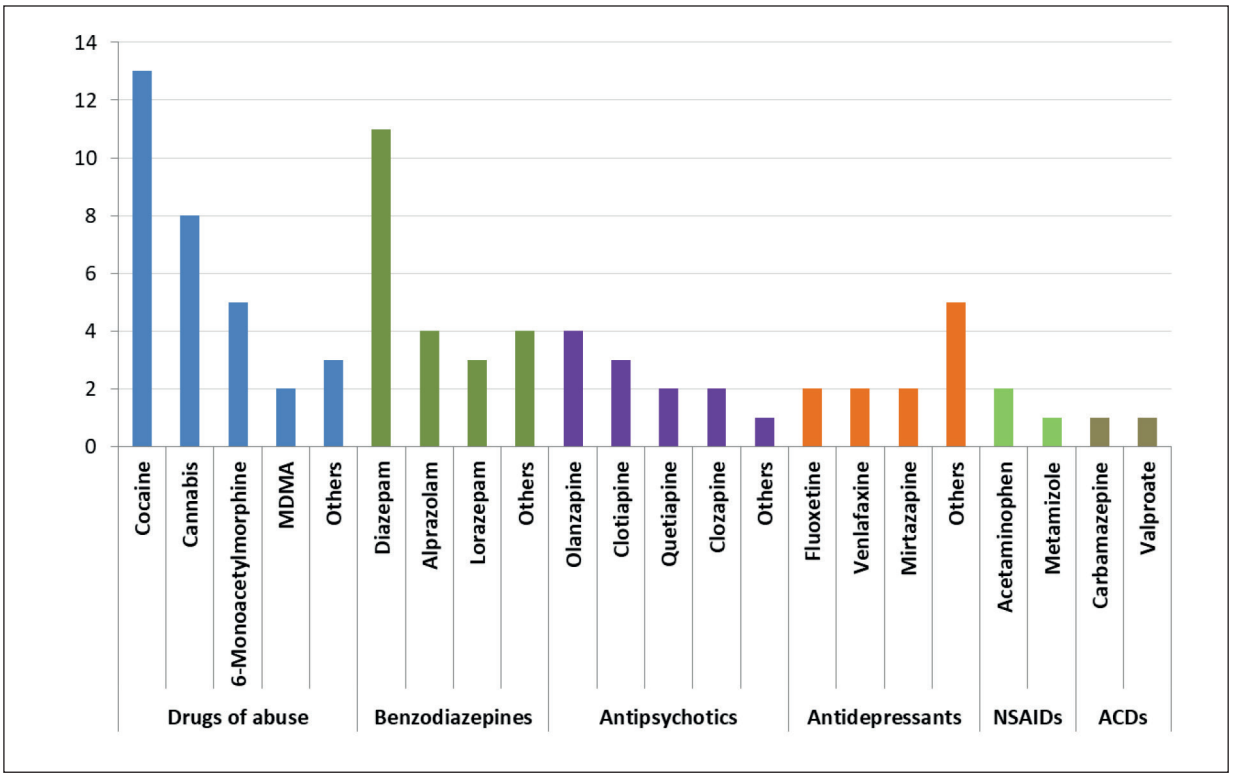

Figure 3. Most common drugs detected in cases with positive toxicology (%) (n=52). NSAIDS: Non-steroidal anti-inflammatory drugs; ACDs: Anticonvulsant drugs.

found substances in positive cases are shown in Figures 2 and 3. These were ethanol (69%), followed by licit drugs (55.7%), and drugs of abuse (38.5%). The most commonly found licit and illicit drugs were diazepam and cocaine, respectively. There were no significant differences between toxic and non-toxic levels of ethanol (n=15 *vs.* n=18; p=0.414). Most licit drugs were at non-toxic levels (n=31 *vs.* n=7; p<0.001), while drugs of abuse were mainly at toxic levels (n=12 *vs.* n=2; p<0.01) (Figure 4).

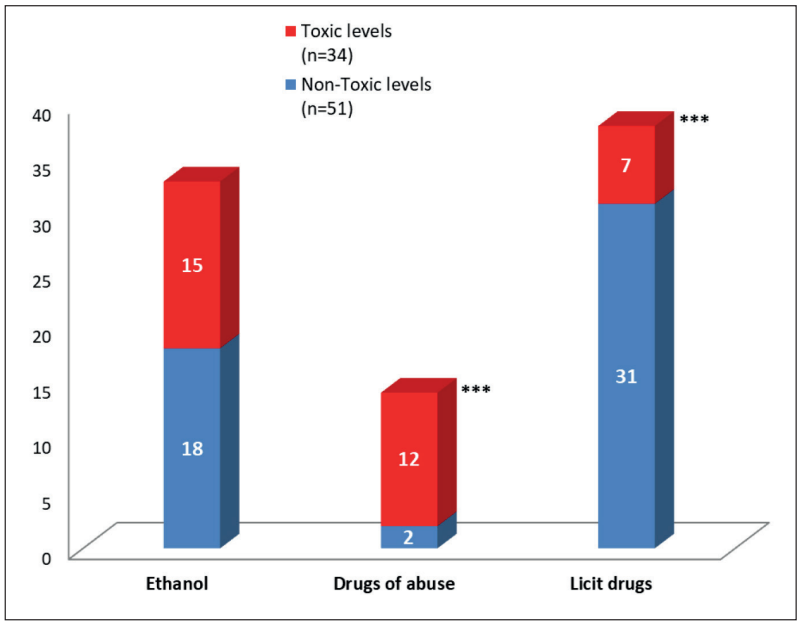

Figure 4. Most common drugs detected grouped according to blood concentrations (n=85). ***p<0.001 between toxic and non-toxic levels (Chi-square test).

Relationships Between Clinical, Autopsy and Genetic Findings with Toxicology Results

Clinical and circumstantial characteristics of SD

Demographic, circumstantial and clinical characteristics of SD cases are shown in Table I. The population was predominately formed by males (84%). The mean age was 39.8 years with a standard deviation (SD) of 8.6 years. Statistically significant differences were found between the groups with positive and negative toxicology depending on the age and the reported comorbidities. Figure 5 shows the increase of SD cases with age. SD cases with positive toxicology were younger than those with negative cases (37.9±9.1 *vs.* 41.9±7.8; p=0.020). In patients who died when aged ≤30 years, positive

Table I. Demographic, circumstantial and clinical characteristics of the SD cases.

	All n=101	Pos. Tox n=52 (%)	Neg. Tox n=49 (%)	p
Age				
Mean±SD	39.8±8.6	37.9±9.1	41.9±7.8	0.020
≤ 30 years	17	13 (25)	4 (8.2)	0.033
> 30 years	84	39 (75)	45 (9.2)	
Gender				
Male	84	45 (86.5)	39 (79.6)	0.351
Female	17	7 (13.5)	10 (20.4)	
Num. comorbidities[a]				
0	17	5 (9.6)	12 (24.5)	0.023
1	17	7 (13.5)	10 (20.4)	0.241
2	17	9 (17.3)	8 (16.3)	0.940
3	12	8 (15.4)	4 (8.2)	0.330
>3	17	14 (26.9)	3 (6.1)	0.017
Not reported	21	9 (17.3)	12 (24.5)	-
Tourist				
Yes	20	10 (19.2)	10 (20.4)	0.879
No	79	41 (78.8)	38 (77.6)	
Not reported	2	1 (1.9)	1 (2.0)	
Place of death				
Home	51	27 (51.9)	24 (49.0)	0.948
Public	24	12(23.12)	12 (24.5)	0.596
Hotel	13	8 (15.4)	5 (10.2)	0.489
At work	4	0 (0)	4 (8.2)	0.101
Hospital	1	1(1.9)	0 (0)	0.279
Not reported	8	4 (8.2)	4 (8.2)	
Activity				
At rest	31	20 (38.5)	11 (22.4)	0.057
Sleeping	6	4 (7.7)	2 (4.1)	0.820
Moderate intensity	7	1 (1.9)	6 (12.2)	0.062
High intensity	6	1 (1.9)	5 (10.2)	0.129
Unknown	51	25 (48.1)	26 (53.1)	-
Witnessed				
Yes	40	20 (38.5)	20 (40.8)	0.578
No	50	28 (53.8)	22 (44.9)	
Not reported	11	4 (7.7)	7 (14.3)	
Season				
Winter	19	8 (15.4)	11 (22.4)	0.364
Spring	23	14 (29.6)	9 (18.4)	0.306
Summer	29	14 (26.9)	15 (30.6)	0.682
Autumn	30	16 (30.8)	14 (28.6)	0.809

Pos. Tox: Positive toxicology; Neg. Tox: Negative toxicology; SD: Standard deviation. [a]**Comorbidities included:** psychiatric disease, history of drug abuse, alcoholism, previous heart disease, cardiovascular risk factors (hypertension, smoking status, diabetes mellitus, obesity, and dyslipidemia), asthma, obstructive sleep apnea, and epilepsy.

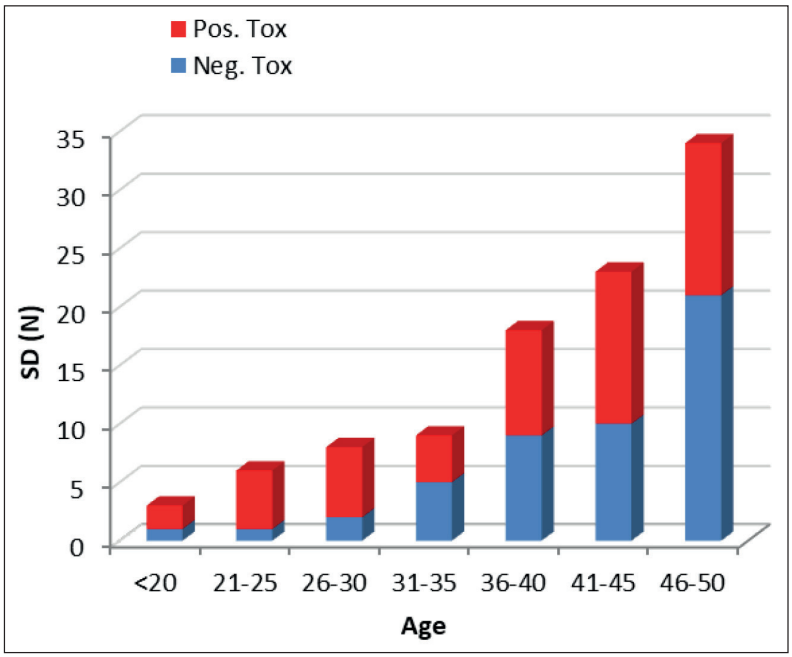

Figure 5. Sudden death according to age group.

toxicology was seen more often than negative toxicology (n=13 (25%) *vs.* n=4 (8.2%); p=0.033). Most SDs without associated comorbidities had negative toxicological results (n=12 *vs.* n=5; p=0.023). By contrast, the deaths with more than 3 comorbidities were associated with positive toxicological results (n=14 *vs.* n=3; p=0.017). Psychiatric disease and/or a history of abuse (drugs or alcohol) were present in 35% SDs. A history of drug abuse and alcoholism was associated with positive toxicological results (n=20 *vs.* n=1; p<0.001 and n=17 *vs.* n=1; p<0.001). The deceased patients were tourists in 19% cases. Places of death were mainly at home and public places (public road, sport center, restaurant, sea, airport**,** and health center).

Autopsy findings

The most common cause of death was SCD (n=49, 48.5%). In SCD and deaths from respiratory causes, the toxicological results were mostly negative (p<0.01 and p=0.042; respectively). In SUD, toxicology results were mainly positive, although the difference was not statistically significant (n=12 *vs.* n=7; p=0.084). In SCD cases (n=49), the first cause of death was ischemic heart disease (IHD) (44.9%), followed by idiopathic left ventricular hypertrophy (ILVH) (12%) and a combination of both, IHD and ILVH (12%). In IHD the toxicology results were predominantly negative toxicology (n=15 *vs.* n=7; p=0.037).

Toxicological findings of SD from toxic causes are shown in Table II. The toxic substances were considered as the first cause or a fundamental cause of death in 25 cases. Main substances detected in blood were: ethanol (70%), cocaine (44%), benzodiazepines (36%), antidepressants (16%), antipsychotics (12%), opiates (12%), cannabis (8%)**,** and MDMA (2%).

Acute poisoning without heart disease was found in 13 cases (52%). Of these, acute ethanol and poly-drug overdose were found in 8 (62%) cases and acute ethanol poisoning were found in 3 cases (23%). The remaining 2 cases (15%) were attributed to drug poisoning without toxic levels.

Cocaine-induced acute coronary syndrome was found in 3 cases (12%).

Acute poisoning with heart disease was found in 5 cases (20%). Of these, 3 were associated with ethanol and poly-drug overdose, 1 with acute ethanol poisoning, and 1 with poly-drug overdose.

Finally, acute poisoning with respiratory or hepatic disease was found in 4 cases (16%). Ethanol and poly-drug overdose were related in 2 cases, acute ethanol poisoning in 1 case**,** and poly-drug overdose in 1 case.

Toxicological findings of SD without toxic cause and positive toxicology are shown in Table II. Toxic substances were not considered as the first cause or a fundamental cause of death in 27 cases. The main substances detected in the blood of these victims were ethanol (56%), benzodiazepines (30%), antipsychotics (26%), antidepressants (11%), cannabis (15%)**,** anticonvulsant drugs (3.7%), and opiates (4%).

Table II. Details and toxicological findings of SD with positive toxicology (n=51).

SD Case	Sex/ age	Cardiac autopsy findings	Comorbidities	Toxicology results	
				Blood (mg/l)	Urine
Acute poisoning without heart disease					
1	M/37	ILVH non-significant	Obstructive sleep apnea	EtOH (positive, <100)[a]; COC (0.04); BZE (0.93); 6-MAM (0.02); MPH (0.3); COD (0.02)	NA
2	M/48	No pathology	HOA; Alcoholism; SS; Obstructive sleep apnea	EtOH (2,970); Quetiapine (0.18); Alprazolam (0.26); Clozapine (0.02)	Quetiapine; Alprazolam; Clozapine
3	F/25	No pathology	HOA; Alcoholism; SS	THC-COOH (0.004)	COC; Amphetamines; Cannabis
4	M/30	No pathology	HOA	EtOH (270); BZE (4.15); EME; NDZP (0.03)	COC; BZE; EME; CE; MPH; 6-MAM; COD
5	F/25	No pathology	HOA; Alcoholism; SS	EtOH (3,200); DZP (0.56); NDZP (2.6); Citalopram (0.54); Temazepam	DZP; NDZP; Citalopram; Temazepam
6	M/26	No pathology	No	EtOH (4,200)	NA
7	M/29	No pathology	No	EtOH (1,760); COC (0.12); BZE (3.20); MPH (0.60)	COC; BZE; EME; CE; MPH
8	M/29	No pathology	PD; Alcoholism; Cardiorespiratory failure; Obesity	EtOH (2,260); Fluoxetine (0.46); Olanzapine (0.06); NDZP (0.30)	Fluoxetine; Olanzapine:NDZP
9	M/38	No pathology	HOA; Alcoholism; SS	EtOH (1,350); COC (0.08); BZE (0.68); Amitriptiline (0.70); Medazepam (0.21); DZP (0.04); NDZP (0.36); Lormetazepam (0.05)	COC; BZE; Amitriptiline; Nortriptiline; Medazepam; DZP; NDZP; Lormetazepam
10	M/40	No pathology	HOA; Alcoholism	Propofol	Propofol; COC[b]
11	M/45	No pathology	PD; Alcoholism; SS	EtOH (710); Venlafaxine (0.2); Mirtazapine(0.1)	Mirtazapine; Clotiapine; Alprazolam
12	M/46	No pathology	Alcoholism; SS; Glottis cancer	EtOH (310)	NA
13	M/34	No pathology	PD; HOA; Alcoholism; SS	EtOH (2,770)	NA
Acute coronary syndrome cocaine induced					
14	M/46	CH; CAD; MI	MI	EtOH (850); COC (0.12); BZE (1.64); EME; CE; Levamisole	COC; BZE; EME; CE; Levamisole
15	M/42	CAD; AOT	HOA; Atrial fibrillation; HT; Diabetes; Obesity; Dyslipidemia	COC (0.09); BZE (0.62); CE	NA
16	M/46	CAD; AOT	HOA	COC (0.66); BZE (0.08); EME; Levamisole	COC; BZE; EME; Levamisole

Continued

Table II (Continued). Details and toxicological findings of SD with positive toxicology (n=51).

SD Case	Sex/ age	Cardiac autopsy findings	Comorbidities	Toxicology results	
				Blood (mg/l)	Urine
Acute poisoning and heart disease					
17	M/26	ILVH	Unknown	EtOH (3,040)	Negative
18	M/25	ILVH	Unknown	EtOH (250); COC (0.04); BZE (1.24); MDMA (0.16); DZP (2.80); NDZP (2.19); Propanolol (6.51)	COC; BZE; EME; CE; MDMA; DZP; NDZP; Propranolol; THC-COOH
19	M/45	IHD	Unknown	THC-COOH (0.001); COC (0.01); EME; Levamisole; Acetaminophen	THC-COOH
20	M/26	HC	Unknown	EtOH (120); COC (0.03); BZE (3.40); EME; CE; MDMA (1.24); Lidocaine	COC; BZE; EME; CE; MDMA; HMMA; Lidocaine; Levamisole
21	M/19	DCM	Unknown	EtOH (Positive, <100)[a]; COC (0.1); BZE (<0.1)	NA
Acute poisoning and hepatic disease					
22	M/32	No pathology	HOA; Alcoholism; Hepatic cirrhosis; SS; HIV; HCV hepatitis; Cholelithiasis	NDZP (0.07); MPH (0.05)	6-MAM; COD; MPH; Temazepam; Cannabinoids
23	M/49	No pathology[c]	PD; Alcoholism; SS; HT; Dyslipidemia	EtOH (2,030); DZP (0.12); NDZP (0.32); Olanzapine (0.46); Lormetazepam (0.01)	NA
Acute poisoning and respiratory disease					
24	M/37	No pathology[d]	HOA; Alcoholism	EtOH (Positive, <100)[a]; NDZP (0.14)	THC-COOH; NDZP; Olanzapine; COD; MPH; 6-MAM
25	M/44	No pathology	Bronchitis Chronic Obstructive; Alcoholism; SS	EtOH (4,120)	NA
Ischemic heart disease					
26	F/42	CAD; AOT; AP	HT; Obesity	ND	Benzodiazepines; Tricyclic antidepressant
27	M/47	CAD; AOT; CH	HT; SS	EtOH (3600)	NA
28	M/43	CAD; S	Alcoholism	EtOH (1070)	NA
29	M/47	CAD; AOT; IHD	HT; SS; Dyslipidemia; migraine	EtOH (110)	NA
30	M/44	CAD; S; MI; HCM	VIH; VHC	EtOH (1130); Alprazolam (0,12)	Alprazolam
31	M/45	Cardiomegaly; CH; M	HT; Obesity	EtOH (<20)	Acetaminophen
32	M/42	CAD; AOT; AP; MI	HOA	EtOH (<20)	THCCOOH
Idiopathic left ventricular hypertrophy					
33	M/38	LVH; MF; CH; AP; RVD	Asthma; SS	Venlafaxine (0,05)	NA
34	M/43	CH; LVD	PD; SS; Dyslipidemia; Obesity	EtOH (<100); Fluoxetine (0,47)	NA

Continued

Table II *(Continued)*. Details and toxicological findings of SD with positive toxicology (n=51).

SD Case	Sex/ age	Cardiac autopsy findings	Comorbidities	Toxicology results	
				Blood (mg/l)	Urine
Other causes					
35	M/47	IHD; Fibro-atomatosis; LVH; MF	PD; Alcoholism; SS; HT; Dyslipidemia; Obesity; enolic hepatopathy; COPD	EtOH (1290); Clorpromazina (0,08)	Clorpromazine; Tiapride
36	M/43	Ascending aorta rupture; HMC; M	Aortic annuloectasia; Paroxysmal atrial fibrillation; SS; alcoholism	Lorazepam (0,03)	Lorazepam
37	F/18	Acute myocarditis	Heart murmur; β-thalassemia minor	EtOH (610)	NA
38	M/50	Right coronary ostium ectopic origin; M	PS; HOA; Alcoholism	Clotiapine (0,05); DZP (0,04) NDZP (0,39); Lormetazepam (0,02); Quetiapine (2,14)	Clotiapine; DZP; NDZP; temazepam; lorazepam; lormetazepam; quetiapine
39	M/49	LVH; MF	PD; Alcoholism; SS	EtOH (380)	Benzodiazepines
40	M/22	No pathology	Epilepsy	Carbamazepine (Positive)	Carbamazepine; THCCOOH
Sudden Unexplained Death					
41	M/37	LVH; MF	HT; Dyslipidemia; alcoholic hepatic steatosis	EtOH (100)	NA
42	M/32	MF; myocytes disorganization	No	THC-COOH	
43	F/22	No pathology	No	EtOH (1340)	NA
44	F/43	CAD	No	EtOH (1900)	NA
45	F/48	No pathology	Congenital coagulopathy	DZP (0,04); NDZP (0,61)	DZP; NDZP
46	M/42	CAD	Unknown	EtOH (<20)	NA
47	M/36	No pathology	PD; HOA; Alcoholism; SS; Asthma	Negative	Alprazolam; MPH
48	M/36	No pathology	PD; HOA; HT; Obesity; Dyslipidemia; Asthma	Sertraline (0,01); Clotiapine (0,04); Clozapine (0,37)	NA
49	M/46	CAD	Unknown	Negative	NDZP
50	M/40	MF	PD; SS; Obesity	Levomepromazine (0,2); DZP (0,04)	Levomepromazine; DZP; NDZP; mirtazapine; olanzapine
51	M/49	AP; hypoplasia	PD; HOA; SS; Obesity	Clotiapine (0,08)	Clotiapine; NDZP; valproate
52	M/31	No pathology	Unknown	EtOH (310)	THC-COOH, clomipramine

AOT, acute occlusive thrombosis; AP, atheroma plaques; ARVC, arrythmogenic right ventricle cardiomyopathy; BZE, benzoylecgonine; CAD, coronary artery disease; CE, cocaethylene; CH, Cardiac hypertrophy; CHD, congenital heart defect; COC, cocaine; COD, Codeine; COPD, Chronic obstructive pulmonary disease; DCM, dilated cardiomyopathy; DZP, diazepam; EME, ecgonine methyl ester; EtOH, ethanol; HCM, hypertrophic cardiomyopathy; HOA, History of abuse; HT, hypertension; IHD, ischemic heart disease; ILVH, Idiopathic Left ventricular hypertrophy; LQTS, long QT syndrome; LV: left ventricle; LVD, Left Ventricular dilatation; LVNC, left ventricle non-compaction; M, myocardiosclerosis; MF: myocardial fibrosis; MI: myocardial infarction; MPH, Morphine; 6-MAM, 6-monoacetylmorphine; NA: Not available; NDZP, Nordazepam; PD, Psychiatric disease; RCM, restrictive cardiomyopathy; RV: Right ventricle; LVD, Left Ventricular dilatation; S: stenosis; SS: Smoking status. [a]Limit of quantification in blood: 100 mg/l. [b]COC, BE, CE and lidocaine detected in hair sample. [c]Hepatic disease: Acute liver failure. [d]Respiratory disease: Pneumonia.

Ischemic heart disease was found in 7 cases (25.9%). 3 cases associated with ethanol, 1 case associated with ethanol and alprazolam, 1 case associated with benzodiazepines and tricyclic antidepressants, 1 case associated with acetaminophen and 1 case associated with cannabis.

Idiopathic left ventricular hypertrophy was found in 2 cases (7.4%) associated with therapeutic drugs.

Other causes were found in 6 cases (22.2%). 3 cases associated with therapeutic drugs, 2 cases associated with ethanol, and 1 case associated with ethanol and chlorpromazine.

SUD was found in 12 cases (44.4%). 6 cases associated with therapeutic drugs, 4 cases associated with ethanol, 1 case associated with ethanol, cannabis and clomipramine, and 1 case associated with cannabis.

Genetic findings

Genetics findings of SD cases are shown in **Supplementary Tables SI, SII, and SIII**.

A genetic study was performed in 70 of 101 SD cases (69.3%). We classified the found genetic variants as pathogenic (P), likely pathogenic (LP), likely benign (LB), benign (B) or of unknown significance (VUS) based on the American College of Medical Genetics and Genomics (ACMG) standards and guidelines for the interpretation of the sequence variants[25]. In addition, we further classified the VUS variants in Likely Benign (LB-VUS), VUS (VUS), and Likely Pathogenic (LP-VUS) using our own criteria based also on the ACMG guidelines and the literature. Under this sub-classification, LB-VUS variants complied with a majority of Benign Supporting criteria. LP-VUS complied with a majority of Pathogenic Supporting criteria. Finally, VUS variants met equally benign and pathogenic criteria or neither. In 28 cases, we could not identify any variants or only benign variants (40% of cases with a genetic study). In the remaining 42 cases we found 12 cases with a clearly P or LP variant (17.1% of cases with a genetic study), and 30 cases with only VUS variants (42.8%). However, if we sub classified the VUS variants, 20 cases had P, LP, or VUS-LP variants (28.5%) and 22 had VUS or LB-VUS variants (31.4%).

If we divided SD cases with a genetic study in three groups: Negative toxicology (N=32), Acute toxicology (N=17) and "Toxics found, but not the cause of the SD" (N=21), we found that P, LP, or VUS-LP variants are evenly distributed (approximately 30%) between the 3 groups. If to these variants we added the VUS variants then we found a trend from: more cases with a potential pathogenic variant in the Acute toxicology group (12/17 cases; 70.5%), Toxics found but not the SD cause (13/21; 62%) to Negative toxicology group (17/32; 53%).

In the groups with 5 or more patients, we detected less genetic variants in patients with "Acute poisoning without heart disease" (4/8; 50%) and with "ischemic heart disease with negative toxicology" (2/5; 40%). While the groups with the most genetic variants were "Acute poisoning and heart disease" with 5/5 (100%) and "positive toxicology with Sudden Unexplained Death" with 8/11 (73%) of patients of the group.

Finally, we compared the cardiac autopsy findings to the known disease associations of the genes in which we found variants. We disclosed that 25/43 (58%) cases in which we found a variant, the gene shows concordance with the heart autopsy findings. In 18/43 of them (42%) the genes with variants were unrelated to the heart autopsy findings.

Discussion

In this study, we found that a quarter of the SDs in the Balearic Islands that occurred in people between 16 and 50 years of age are directly caused by toxic substances. In addition, more than half of all cases showed positive toxicological findings. To the best of our knowledge, the present study is the first in which the complete toxicological profile of SD cases has been assessed and related with the clinical, autopsy, and genetic findings. The vast majority of similar studies published so far do not include toxicological information of the cases.

Impact of the Toxicological Results Found in Cases of SD

Half of the investigated cases had positive toxicology findings. Toxic substances were considered the first cause or a fundamental cause of death in a quarter of the SD cases analyzed. These facts reinforce that alcohol and drugs are important factors in SD. Accordingly, comprehensive toxicological analysis is mandatory when investigating these deaths.

SD caused by toxic substances was associated with acute poisonings in men, mainly by ethanol and/or cocaine alone or in combination with other

drugs of abuse or licit drugs, which probably contributed to their known cardiovascular toxicity. In addition, in 20% of acute poisoning cases, there was a previous heart disease that likely played a decisive role in the fatal outcome of cases.

In almost all SDs caused by toxic substances, blood levels of alcohol and/or drugs were in the toxic range. In a few cases, the detection of toxics in urine or humour vitreous along with medical history and other evidence of the autopsy allowed the diagnosis of acute poisoning as a cause of death, in spite of blood levels not being in the toxic range.

SUD cases had mostly positive toxicology results, but none of them had a toxicological profile that the forensic pathologists concluded could explain their death. These findings were similar to those of other studies performed in Denmark in people aged between 1 and 49 years[10,26].

Poypharmacy was found in 12% of SDs and two or more substances were detected in most cases (69%). Previous findings support these results[10,26]. This suggests a substantial element of mixed intoxication or drug interactions between psychotropic drugs and other medicines when drug concentrations were in a therapeutic range[27,28].

Figure 3 reveals that ethanol is frequently involved in SD, being present in 69% of all toxicology-positive cases. This result is not unexpected. The frequency reported is higher than those of previous studies of SCD performed in Denmark (ethanol found in 35% and 17% of positive cases), but it is similar to the detection frequencies of ethanol in forensic autopsies[10,26,27].

After ethanol, the most frequently detected classes of substances were drugs of abuse, benzodiazepines, antipsychotics, and antidepressants (Figure 3).

Cocaine is the most commonly detected drug of abuse. It was found in 13% of all of the SDs studied. This result is higher than the 3.1% and 9% previously reported in two studies performed in Spain[9,29]. It is known that cocaine causes more cardiovascular complications than any other illegal drug[15]. Both acute and chronic cocaine use may cause arterial hypertension, aortic dissection, arrhythmias, acute pulmonary edema, cardiomyopathy, and SD. The association between acute coronary syndrome (ACS) and cocaine consumption was shown by Coleman et al[30] in the early 1980s. This association has significantly increased over the past few years[31]. ACS was present in three of the SDs caused by toxic substances in our study. On the other hand, concomitant cocaine and ethanol consumption determines the production of an active metabolite, cocaethylene, which is more toxic than cocaine or ethanol alone[15]. In our study, ethanol was present in 69% of SD cases that were cocaine positive, a percentage that is similar to that which was previously reported[29].

The high rate of detection of benzodiazepines in SD cases has been previously reported, but they were generally not considered to have cardiotoxic characteristics[10,27]. However, SD caused solely by benzodiazepines has been reported[32]. Further, in futures studies new designer benzodiazepines that have reached the illegal drug market over the past years, should be taken into account, especially because little is known about the risks related to their use[32,33].

The frequent detection of antipsychotics and antidepressants is not surprising considering that this study included a large number of individuals with psychiatric disorders. The use of psychotropic drugs, especially the combined use of antipsychotic and antidepressant drugs, is strongly associated with an increased risk of SCD[34]. As previously reported, blood levels of psychotropic drugs were mostly at non-toxic concentrations[10]. Nevertheless, there is some evidence that the increased risk of SD occurs even at moderate doses[35].

Relationships Between Clinical, Pathological and Genetic Findings with Toxicology Results

Clinical and circumstantial characteristics of SD

In our cases, the SDs mainly occurred in men with an incidence that increased with age, according to the findings of other studies[2,5,10,26]. Patients with positive toxicological results were younger than those with negative results. Considering age globally and using the 30-year cut-off, these results probably differ from other studies due to the fact that they excluded the toxicological origin of the cause of SD and the highly variable age criteria for the populations studied[10,36-39]. As expected, psychiatric disorders, history of abuse, and alcoholism showed higher proportions of positive toxicology results[10,40]. Dual diagnosis of concurrent mental and substance abuse disorders is widespread with 13.9% of SDs in the present study having an overlapping history of both.

Autopsy findings

As expected and considering the middle-age of the majority of the population included in the study, SCD (45% IHD) and SUD were the most common causes of SD according to previous studies[2,5,6,27,41]. Toxic substances were considered the first cause or a fundamental cause of death in a quarter of the SD cases analyzed. This result was higher than others reported in a study performed in Ireland in people aged between 14 and 35 years, where drug-related or alcohol-related SD accounted for 6.5% of cases[41].

Genetic findings

The genetic findings in our study confidently explain the causes of SD in a small portion of cases (24%). For example, in the "Toxics Found but not the Cause" group case number 36, a variant was detected in the SMAD3 gene which is associated to Loeys-Dietz type 3 and the autopsy found a rupture of the ascending aorta which would be clearly related to this disease. In other cases we disclosed a clear concordance between genes that cause HCM or DCM and autopsy findings (for example, cases 14 and 20, "SD with toxic cause" group, cases 33 and 34 in "SD without toxic cause and positive toxicology" group, and cases 16, 29, and 44 in "SD cases with negative toxicology" group).

However, the majority of variants and genes do not provide or provide uncertain explanations. The reason for this may stem from the imperfect knowledge of the genotype-phenotype correlations and on although genetically triggered, undetectable events after death. This would be the case in 20 patients (48% of cases) in which we found mutations that cause arrhythmias that are not easily detected during the autopsy and thus they still could be related to the cause of death. For example, case 40 of the "Toxics Found but not the Cause" group had no cardiac autopsy findings but the patient presented epilepsy and was carbamazepine positive. This patient had variant in the KCNQ1 gene, a gene linked both to epilepsy and sudden death[42]. And in case 19 of the "SD with toxic cause" group there was a variant in the KCNE1 gene that is associated to drug induced LQTS and the case showed an ischemic heart disease, and acute poisoning by THC-COOH, cocaine, EME, acetaminophen, and levamisole[43]. Despite these cases, the direct relation between genetic variant-gene-cause of death (toxic or autopsy findings) was not always evident.

On the other hand, mutations were detected in all SD cases with acute poisoning and heart disease (5/5) and in SD with acute coronary syndrome cocaine induced (2/2). These findings could suggest that the relationship between drug use and genetic predisposition in the death, as previously, has been reported in a SCD in a patient with arrhythmogenic right ventricular cardiomyopathy under cocaine and alcohol effects[44]. Thus, genetic factors may be important variables independently if there is a probable toxic cause of SD or not, as they would act by lowering the threshold to suffer an event of SD triggered by toxics or other environmental circumstances.

Finally, in order to prevent future events, forensic research can improve the understanding of the association between SD and toxic substances[16]. To achieve this goal, laboratories need advanced technology and highly and standardized general unknown screenings and targeted strategies. Determination of highly potent opioids, such as fentanyl derivatives and NPS represent a challenge. Also, solvent/volatiles and poppers should be targeted. This focused research could be enhanced through the cooperation between forensic pathologists and laboratories of forensic toxicology with cardiologists, psychiatrists, geneticists, and laboratories of clinical toxicology[44-48].

Our study presented several limitations. First, information about demographic characteristics and the clinical circumstances of some SD was exiguous because the data were provided by coroners, often after collecting it, at the time of death that was probably not the best moment for interviewing family. In other cases, the deceased were tourists, meaning that the medical history was unknown. Second, it could be speculated that non-investigated drugs, such as performance-enhancing substances, new therapeutic drugs, or new psychoactive substances, could have contributed to the SDs. For these reasons, no new toxic drugs have been detected in our study. A new project is designed to overcome these limitations. Third, the SDs which occurred in Menorca and Ibiza/Formentera were included in the MUSIB program progressively throughout the period of this study. Therefore, the level of SDs studied was lower than the number that occurred on these islands.

Conclusions

This study illustrates that a large fraction of young and middle-aged adults SD victims present positive post-mortem toxicological findings and a quarter of them were directly caused by the use of toxic substances.

Systematic toxicological investigation represents valuable information that impact on epidemiological data and on an unexposed issue regarding young victims of SD in our country. The identification of the factors that trigger SD provides a good approach to contribute to the implementation of the targeted interventions aimed at improving healthy lifestyle and therapeutic measures to avoid future episodes based on individual and family risk profiles.

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Conflict of Interests

The authors declare they have no conflict or financial interests.

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