

Strontium Levels in Different Causes of Death: Diagnostic Efficacy in Drowning

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Abstract Trace element determination can be applied in forensic medicine to diagnose the cause of death. Drowning is the second leading cause of death from unintentional injury. Despite the many diagnostic methods used, the post-mortem diagnosis of drowning continues to be one of the most difficult in forensic pathology. Strontium is a highly sensitive marker of water aspiration in a liquid medium rich in this metal. The aims of this study were to confirm the diagnostic value of strontium in cases of drowning compared with other causes of death, to analyse factors that could affect its concentration and to ascertain the sensitivity and specificity of strontium in right and left ventricles and peripheral serum for the post-mortem diagnosis of drowning. We studied 120 cadavers selected from medico-legal autopsies with different causes of death. Strontium (Sr) levels were measured in the serum (left and right ventricles and peripheral vein) of all cadavers and, in the case of drowning, in the water medium itself, by using Zeeman AAS. Our results confirm the usefulness of blood Sr levels for diagnosing seawater and freshwater drowning, although great care should be exercised in the latter case.

Keywords Strontium · Forensic medicine · Biochemistry · Drowning

Introduction

Trace element determination can be applied in forensic medicine to diagnose the cause of death. Drowning is a major, but often neglected, public health problem. However, the lack

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of a uniform and internationally accepted definition has prevented suitable epidemiological data from being collected on drowning incidents. For this reason, in the first World Congress on Drowning (WCOD-Amsterdam 2002), a definition was finally agreed: “Drowning is the process of experiencing respiratory impairment from submersion/immersion in liquid”, which ensures that all victims of drowning have some important unique characteristic, respiratory impairment due to exposure of the patient’s airway to liquid, regardless of the result (death or survival) [1].

Drowning is the second leading cause of death from unintentional injury, after road traffic injuries [2], and accounts for more than half a million deaths annually worldwide. This number is probably a gross underestimate because of underreporting [3]. There may be large variations in time and place because drowning may be related to several types of daily and/or recreational activities (swimming, fishing, boating), various types of exposure to water (oceans, lakes, rivers, swimming pools, bathtubs) and other risks factors, some controllable and others unpredictable (alcohol and drugs use, behavioural risks factors, natural catastrophes, etc) [1].

Death by drowning presents some interesting questions in forensic medicine. As Modell et al. said, to attribute drowning as the cause of death in the case of any body retrieved from the water, without providing evidence of water aspiration, is a risky diagnosis [4]. Despite the many diagnostic methods used, both biological and thanato-chemical, the post-mortem diagnosis of drowning continues to be one of the most difficult in forensic pathology. The ideal diagnostic test as definite proof for drowning still needs to be established, and more research is necessary [5].

Among the many chemical markers studied as possible markers for drowning, strontium in blood or serum has been used by several authors [6–15] since its level always increases in arterial blood as a result of both seawater and freshwater drowning. The change observed in the strontium concentration of arterial blood after drowning is almost exclusively the consequence of strontium being absorbed from the water medium into arterial blood. Strontium is a common component of marine salts and is found in concentrations of around 8,000 µg/l, the exact value depending on the degree of salinity. However, its concentration in rivers varies enormously, and its determination in the case of freshwater drowning is not always valid for determining the cause of death.

The aims of this study were to confirm the diagnostic value of strontium in cases of drowning (fresh and saltwater drowning) compared with other causes of death, to analyse factors that could affect its concentration and to ascertain the sensitivity and specificity of strontium in right and left ventricles and peripheral serum for the post-mortem diagnosis of drowning.

Material and Methods

Forensic Autopsy Cases

We studied 120 cadavers (103 men and 17 women) selected from medico-legal autopsies performed in the Institute of Forensic Medicine, Cádiz (South of Spain). The study was approved by the Ethics Committee of the Institute of Forensic Medicine. The mean age of the subjects was 48.73 years (SD 19.45; range 2–86 years), while the mean post-mortem interval was 30 h (SD 39.59; range 3–216 h).

According to the scene, cause and circumstances of death, together with autopsy findings (external foam, frothy fluid in airways, overlap of the medial edges of the lungs),

cases were classified into groups as follows: (a) drowning ($n=47$), (b) other asphyxias ($n=44$) and (c) other causes ($n=29$). Also, the cases were sub-classified into seven groups: (1) freshwater drowning ($n=15$), (2) seawater drowning ($n=32$), (3) hanging ($n=33$), (4) gunshot ($n=8$), (5) jumping from a height ($n=17$), (6) other asphyxias ($n=11$) and (7) other causes ($n=4$). The age of the victims and post-mortem intervals (mean hours \pm SD) for the groups are shown in Table 1.

Methods

Following the sample-collecting method proposed by Azparren et al. [13] for cases of drowning, blood samples from the right and left ventricles and the femoral vein were obtained in the autopsies using standard techniques before being stored at -70°C . Strontium (Sr) levels were measured in the serum of all cadavers and, in the case of drowning, in the water medium itself, by using Zeeman AAS (AAnalyst 600) Perkin-Elmer instrument in the Biochemistry Department of University Hospital “Virgen de la Arrixaca” (Murcia, Spain). The sensitivity, detection limit and linear range was 0.36, 4.6 and 10–40 $\mu\text{g/l}$, respectively. A total of 407 strontium determinations were made (360 in serum and 47 in water medium).

Statistical Analysis

For statistical analyses of the data, the SPSS 14.0 software was used. Descriptive analysis of the data (mean, median, standard deviation and range), correlation (Spearman correlation) between different variables and discriminant analysis were carried out. The Kruskal–Wallis test, a non-parametric test for more than two independent samples, was used to compare groups. Also, specific contrasts for each variable grouped according to diagnostic category were carried out using the non-parametric Mann–Whitney test for two independent samples. A probability level of $P \leq 0.05$ was considered significant.

For each of the concentrations of strontium studied, a receiver operating characteristic curve (ROC) was drawn, and the area under the curve was measured using a non-parametric test. The diagnostic performance of a test or the ability of a test to discriminate between drowning cases and normal cases was evaluated using ROC curve analysis. The

Table 1 Age and Post-mortem Interval (in Hours)

Groups	Samples	Percent	Age (years old)		Post-mortem interval (h)	
			Mean	SD	Mean	SD
Drowning	47	39.2	48.9	18.9	36.0	44.0
Other asphyxias	44	36.7	50.4	20.2	31.1	46.0
Other causes	29	24.2	45.8	19.4	18.5	6.0
Total	120	100	48.7	19.4	30.0	39.5
Freshwater drowning	15	12.5	47.2	22.2	37.0	54.3
Seawater drowning	32	26.7	49.7	17.4	35.5	39.2
Hanging	33	27.5	50.0	20.9	24.3	35.7
Gunshot	8	6.7	34.5	13.7	15.5	4.6
Jumping from a height	17	14.2	53.1	16.6	20.2	4.6
Other asphyxias	11	9.2	51.7	18.6	51.0	66.2
Other causes	4	3.3	37.5	29.5	17.0	11.1

Mean and standard deviation (SD) values for the groups

cut-off point for each variable was taken as the point nearest the “ideal” point of the ROC curve in which sensitivity would be 1 and 1-specificity would be 0.

Results

No statistically significant differences were observed in age or post-mortem interval according to “cause of death” or “diagnostic group”. Figure 1 shows the mean Sr levels in the right and left ventricles, peripheral serum, and differences in Sr levels in the left and right ventricles (LvSr–RvSr) in drowning and other causes of death. Table 2 shows the values for Sr levels in the water medium and length of time the body had been in the water for cases of fresh and seawater drowning. Table 3 shows the values (mean, standard deviation, median and range) obtained for Sr in right and left ventricles and peripheral serum for the groups. Statistically significant correlations were observed between the Sr levels in the water medium and those found in the right ventricle ($P=0.040$), left ventricle ($P=0.005$), peripheral serum ($P=0.040$) and LvSr–RvSr ($P=0.006$).

Using a non-parametric test (Kruskal–Wallis test), statistically significant differences in Sr levels were observed in right and left ventricles and peripheral serum between the diagnostic group and cause of death (Table 4). Also, in water medium, we found statistically significant differences in Sr concentrations between seawater and freshwater drowning. The highest strontium levels were obtained in the group, which had died from drowning, and, of these, the highest levels were observed for seawater drowning.

No statistically significant correlations were observed between Sr levels in either ventricles and peripheral serum and the age and post-mortem interval. We found statistically significant correlations between the variable cause of death (seven categories

Fig. 1 Mean strontium levels in the right and left ventricles, peripheral serum and differences in strontium levels in the left and right ventricles (LvSr–RvSr) in drowning and other causes of death

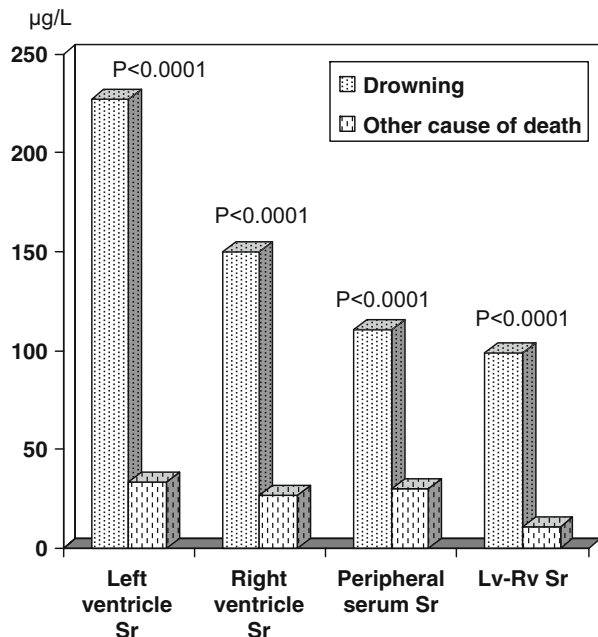


Table 2 Mean, Standard Deviation (SD), Median and Range Value of Strontium Level in Water Medium and Time in the Water in Freshwater and Seawater Drowning

	Strontium in water medium ($\mu\text{g/l}$)				Time in the water (h)			
	Mean	SD	Median	Range	Mean	SD	Median	Range
Freshwater drowning	1,727.5	1,117	1,212.5	520–3,750	22.4	68.0	0.5	0–216
Seawater drowning	5,646.5	2,092.1	4,825	2,700–10,200	23.4	41.5	3.0	0–168
Total	4,615.1	2,560.5	4,400	520–10,200	23.2	47.52	1.0	0–216

established) and right Sr ($P \leq 0.0001$), left Sr ($P \leq 0.0001$) and femoral vein Sr level ($P \leq 0.0001$). Statistically significant correlations were also found between the variable diagnostic group (three categories established) in Sr concentrations in right ventricle ($P \leq 0.0001$), left ventricle ($P \leq 0.0001$) and peripheral vein ($P \leq 0.0001$).

The Mann–Whitney test (Table 5) pointed to differences in Sr levels between drowning and other asphyxias and other causes in right and left ventricles and peripheral vein. Also, in the case of cause of death, statistically significant differences existed in Sr levels between freshwater drowning and all other causes of death in right and left ventricle serum, but in femoral serum, only between freshwater and seawater drowning and hanging. In seawater drowning, we found statistical differences in right and left ventricles and peripheral vein between all causes of death.

No statistically significant differences were found according to sex in any of the sampled sites.

For the discriminant analysis, we used the diagnostic group as the grouping variable, establishing two groups, deaths from drowning (47 cases) and other causes of death (73 cases), and taking the levels of Sr in heart as independent variables. Correct classification was found in 81.5% of the cases (98.3% in the group of non-drowning and 56.1% in the group drowning).

We found statistically significant correlation between length of time in the water and Sr concentration in the right ventricle ($P = 0.012$). However, when the four cases in which the cadaver had spent more than 72 h in water were eliminated, no significant correlation existed between these variables.

We considered the ROC curve expressed by concentrations of Sr in ventricles and peripheral serum, paying special attention to the area (Table 6), which represents the correct diagnosis in two deceased individuals, one through drowning and the other through other cause. Figure 2 shows the ROC curve of Sr in the different sites studied.

Discussion

Drowning has been a controversial subject in medico-legal publications, with some interesting revisions published on the theme [5]. Analysing the drowning process by extrapolating experimental animal models to the drowning process in human does not seem to be helpful because while human drowning leads to an aspiration of only 2–4 ml water/kg, in animal experiments, more than 10 ml water/kg will be artificially aspirated [16], so it is essential to look at real cases of human drowning.

Strontium is a highly sensitive marker of water aspiration in a liquid medium rich in this metal, and for this reason, it is considered a good diagnostic indicator of drowning, especially in seawater. The mean levels of Sr in healthy persons ranged from 5.7 to 15.6 $\mu\text{g/l}$, which may

Table 3 Mean, Standard Deviation (SD), Median and Range Value for the Strontium (Micrograms per Litre) in Right and Left Ventricles and Peripheral Serum in the Diagnostic Groups and Causes of Death

Groups	Right ventricle Sr ($\mu\text{g/l}$)			Left ventricle Sr ($\mu\text{g/l}$)			Peripheral vein Sr ($\mu\text{g/l}$)			LySt-RvSr ($\mu\text{g/l}$)						
	Mean	SD	Median	Range	Mean	SD	Median	Range	Mean	SD	Median	Range	Mean	SD	Median	Range
Drowning	150.1	160.5	102	9–714	227.6	263	102.5	17.5–1,198	110.9	110.2	71.0	16.5–590	98.9	139.4	27.5	0.5–552
Other asphyxias	28.6	24.0	21.5	5.5–120	34.3	27.8	25	10–160	30.1	21.1	23.2	5.5–101	11.7	13.4	7	0–50
Other causes	24.5	14.8	19.0	10–71	31.8	22.1	24.2	6–107	30	14.8	26	13–82	9.6	10.8	6.5	0–45.5
Total	73.7	116.2	30.2	5.5–714	110.6	191.4	38.5	6–1,198	62.3	80.9	32.5	5.5–590	46.56	98.8	11.5	0–45.5
Freshwater drowning	44.8	41.6	35	9.5–142	47.5	33.5	35	20–127	36.8	35.8	25	16–131	13.0	6.4	14	3.5–24.5
Seawater drowning	179.8	169.4	123	15–714	278.2	277.3	165	17–1,198	131.7	115.3	97.2	21–590	123.0	149.4	58.5	0.5–552
Hanging	28.8	22.2	21.5	9.5–120	31.2	17.1	24.5	13–84	28.3	20.2	22	5.5–101	10.1	10.7	6.7	0–37
Gunshot	29.8	20.9	20.5	10–71	29.9	26.3	23	6–80.5	31.2	12.7	29.5	13.5–50.5	6.2	3.9	6.5	1.5–11.5
Jumping from a height	23.3	12.9	18.7	12–61.5	33.4	23.1	25	13.5–107	31.5	17.1	25	13–82	10.7	11.54	7.2	0–45.5
Other asphyxias	28.3	29.8	19	5.5–110	46.5	44.3	29.2	12–160	36.1	22.8	34	9–84	18.4	18.8	11	1–50
Other causes	18	3.2	18	14–22	18.6	6.0	19.5	10.5–25	20.1	5	22	14.5–24	2.3	1.0	2.2	3.5–2.2

Table 4 Kruskal–Wallis Test Used for the Strontium Levels (Micrograms per Litre) in the Diagnostic Group and Cause of Death

Variable	<i>df</i>	Statistic	Probability
Diagnostic group			
Right ventricle	2	40.940	<0.0001
Left ventricle	2	42.972	<0.0001
Femoral vein	2	31.790	<0.0001
LvSr–RvSr	2	7.193	0.027
Cause of death			
Right ventricle	6	13.946	<0.0001
Left ventricle	6	45.704	<0.0001
Femoral vein	6	47.486	<0.0001
LvSr–RvSr	6	19.209	0.004
Water medium	1	39.611	<0.0001

Diagnostic group = (a) drowning ($n=47$), (b) other asphyxias ($n=44$) and (c) other causes ($n=29$)

Cause of death = (1) freshwater drowning ($n=15$), (2) seawater drowning ($n=32$), (3) hanging ($n=33$), (4) gunshot ($n=8$), (5) jumping from a height ($n=17$), (6) other asphyxias ($n=11$) and (7) other causes ($n=4$)

LvSr–RvSr = The difference between the Sr concentrations of blood from the left and right ventricles

vary depending on the type of drink and food consumed [8] and probably the use of strontium ranelate to treat osteoporosis [17].

The mean range of Sr in blood in causes of death other than drowning is 11.9 ± 1.36 $\mu\text{g/l}$ according to Piette et al. [8] and 28.0 $\mu\text{g/l}$ (SD: 12.4; range 14.5–47.5) according to Azparren et al. [13]. In our study for causes of death other than drowning, the mean level in serum depended on the sampling site: 26.95 $\mu\text{g/l}$ (SD 20.67) in right ventricle, 33.32 $\mu\text{g/l}$ (SD 25.43) in left ventricle, 30.20 $\mu\text{g/l}$ (SD 18.60) in peripheral serum and 10.79 $\mu\text{g/l}$ (SD 12.29) in LvSr–RvSr, levels similar to those described by Azparren et al. [13]. In agreement with the same author, Azparren et al. [15], our results establish that neither age nor gender significantly affect Sr levels.

In the diagnosis of drowning, Azparren et al. [10, 11] point to the usefulness of the differences in Sr levels between left and right ventricles, considering difference in excess of 70 $\mu\text{g/l}$ between the left and the right sides of the heart (LvSr–RvSr) typical of seawater drowning, or Sr levels in the left ventricle exceeding 172 $\mu\text{g/l}$, which is less likely to be the subject of analytical error [15]. In our study, these parameters are fulfilled in 50% of the cases of seawater drowning.

We obtained statistically significant differences in Sr levels in the right and left ventricles and the peripheral serum and differences in the concentrations between left and right ventricles (LvSr–RvSr), depending on the diagnostic group, the levels being higher in drowning than in other asphyxias or other causes of death, findings that reflect those of other authors [7, 9, 10]. Also, there were statistically significant differences in strontium levels when the different causes of death were analysed: The highest levels occurring in seawater drowning, followed by cases of freshwater drowning. When the seawater cases were analysed independently and compared with other causes of death, significant differences in Sr were found in the left and right ventricles, peripheral serum and LvSr–RvSr.

On the other hand, it is necessary to bear in mind factors other than cause of death that may modify Sr levels in blood, such as length of time in the water since, although there is very little evidence to suggest the possibility of post-mortem diffusion from water to blood,

Table 5 Mann–Whitney Test Used to Compare Mean Values of the Strontium According to the Diagnostic Group and Cause of Death

Variable	Groups	Probability	
Right ventricle	a–b	<0.0001	
	a–c	<0.0001	
	1–2	0.012	
	1–3	0.007	
	1–4	0.02	
	1–5	0.005	
	1–6	0.02	
	1–7	0.02	
	2–3	<0.0001	
	2–4	<0.0001	
	2–5	<0.0001	
	2–6	<0.0001	
	2–7	0.02	
	Left ventricle	a–b	<0.0001
a–c		<0.0001	
1–2		0.02	
1–3		0.001	
1–4		0.02	
1–5		0.008	
1–6		0.02	
1–7		0.02	
2–3		<0.0001	
2–4		<0.0001	
2–5		<0.0001	
2–6		<0.0001	
2–7		0.001	
Femoral vein		a–b	<0.0001
	a–c	<0.0001	
	1–2	0.005	
	1–3	0.014	
	2–3	<0.0001	
	2–4	<0.0001	
	2–5	<0.0001	
	2–6	<0.0001	
	2–7	0.03	
	LvSr–RvSr	a–b	<0.0001
		a–c	<0.0001
		1–2	<0.0001
		1–7	0.003
		2–3	<0.0001
2–4		<0.0001	
2–5		<0.0001	
2–6		0.001	
2–7		0.001	

Diagnostic group = (a) drowning ($n=47$), (b) other asphyxias ($n=44$) and (c) other causes ($n=29$)

Cause of death = (1) freshwater drowning ($n=15$), (2) seawater drowning ($n=32$), (3) hanging ($n=33$), (4) gunshot ($n=8$), (5) jumping from a height ($n=17$), (6) other asphyxias ($n=11$) and (7) other causes ($n=4$)

LvSr–RvSr = The difference between the Sr concentrations of blood from the left and right ventricles

Table 6 Areas Below the ROC Curves, Standard Error and Lower and Upper Limits of the Area

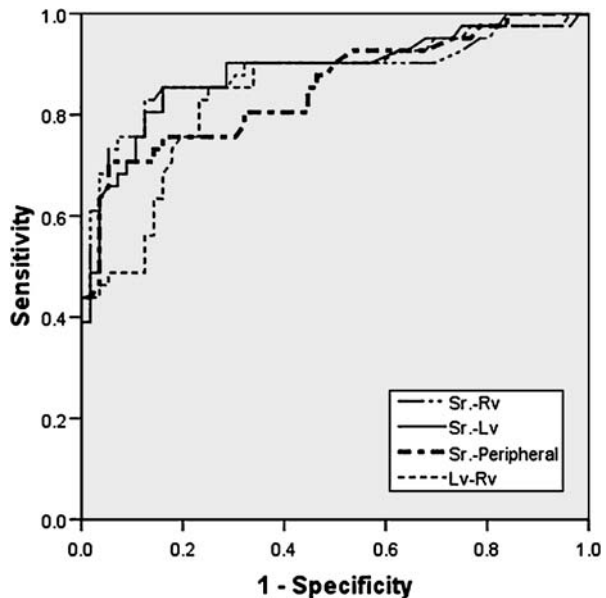
Variable	Area	Standard error	<i>P</i>	Lower limit	Upper limit
Drowning					
Strontium right ventricle ($\mu\text{g/l}$)	0.882	0.040	<0.0001	0.803	0.961
Strontium left ventricle ($\mu\text{g/l}$)	0.886	0.037	<0.0001	0.814	0.958
Strontium peripheral serum ($\mu\text{g/l}$)	0.852	0.041	<0.0001	0.772	0.933
Strontium LvSr–RvSr ($\mu\text{g/l}$)	0.844	0.042	<0.0001	0.763	0.926
Seawater drowning					
Strontium right ventricle ($\mu\text{g/l}$)	0.938	0.030	<0.0001	0.879	0.996
Strontium left ventricle ($\mu\text{g/l}$)	0.941	0.029	<0.0001	0.883	0.998
Strontium peripheral serum ($\mu\text{g/l}$)	0.935	0.028	<0.0001	0.880	0.990
Strontium LvSr–RvSr ($\mu\text{g/l}$)	0.892	0.041	<0.0001	0.812	0.973

we found a significant correlation between this time and Sr levels in the right ventricle. However, when the four cases, which remained more than 3 days in the water, were eliminated, the significant correlation between both variables disappeared, which does indeed suggest that there was post-mortem diffusion of Sr, lending weight to the usefulness of Sr as a marking of drowning only within the first 3 days of death [13]. However, information regarding the time in water is not always possible.

Freshwater drowning provides a disparity of results as regards Sr concentrations. For example, Piette et al. [7] observed an overlapping in a range of Sr levels between drowned and non-drowned subjects, while Fornes et al. [9] found no such overlapping. Azparren et al. [13] claimed that only 32% of freshwater drowning could be diagnosed by determining Sr levels in blood.

In our study, the highest levels of Sr for freshwater drowning were found in left and right ventricles, with significant differences from the rest of the causes of death. However, in

Fig. 2 The ROC curve of strontium in the right and left ventricles, peripheral serum and differences in strontium levels in the left and right ventricles (LvSr–RvSr)



peripheral serum, differences in Sr levels only permitted seawater drowning and hanging to be differentiated. As regards the differences between left and right ventricles (LvSr–RvSr), significant differences were only found between seawater drowning and other causes (lowest levels).

Taking into consideration the cases of drowning (sea and freshwater), the discriminant analysis confirms the high negative predictive capacity for excluding death due to drowning, in which there is 81.5% correct classification, which rises to 97.2% in subjects who did not die from drowning. This means that only two cases of the 73 subjects who died for reasons other than drowning were badly classified, showing levels of strontium comparable to those who had died from drowning.

In the ROC curve analysis, it can be seen that all the areas representing strontium in heart (both ventricles), peripheral serum and LvSr–RvSr are significantly different from 0.50 for drowning in general and seawater drowning but not for freshwater drowning. Strontium levels in fresh and seawater cases considered together, show an area greater than 0.85. According to our results, cut-offs lower than those previously proposed [12] show a good diagnostic capacity with sensitivities of above 90% and specificity above 80% (Table 7).

Conclusion

Our results confirm the usefulness of blood Sr levels for diagnosing seawater and freshwater drowning, although great care should be exercised in the latter case. We agree with Azparren et al. [13] that Sr should be measured in distant sites in the blood stream such as the left and the right ventricles.

Although more evidence is needed, we think that post-mortem diffusion of Sr takes place in water and so, Sr analysis should only be used in cadavers which have been in the water for less than 72 h.

Whatever the case, the analysis of Sr should be considered a complementary tool for diagnosing drowning, and in no case should it substitute a combination of morphological (macroscopic and histological) findings and circumstances of death, as well as other techniques.

Table 7 Cut-off Points Established According to the use of a Receiver Operator Characteristic Curve

Variables	Cut-off point	Specificity of the cut-off point	Sensitivity of the cut-off point
Drowning			
Strontium right ventricle ($\mu\text{g/l}$)	33.75	83.9	85.4
Strontium left ventricle ($\mu\text{g/l}$)	51.25	87.5	85.7
Strontium peripheral serum ($\mu\text{g/l}$)	38.75	83.9	75.6
Strontium LV–RV ($\mu\text{g/l}$)	13.75	76.8	82.9
Seawater drowning			
Strontium right ventricle ($\mu\text{g/l}$)	51.25	89.2	90.6
Strontium left ventricle ($\mu\text{g/l}$)	51.25	84.6	93.8
Strontium peripheral serum ($\mu\text{g/l}$)	41.25	86.2	90.6
Strontium LV–RV ($\mu\text{g/l}$)	18.15	83.1	90.6

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