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Sex- and age-related composition of 10 617 calculi analyzed by infrared spectroscopy

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Abstract A series of 10 617 calculi were analyzed by stereomicroscopy and infrared spectroscopy. This first study of French calculi was compared with large series in the literature. That the frequency of pure calculi was the lowest ever observed can be related to the methodology routinely used in our laboratory, which includes microsampling. We described more than 70 components among the 10 617 calculi. The overall sex ratio male to female patients was high (2.27) and increased over the period 1981–1993. Calcium oxalate was the most frequent component (86.48%), followed by calcium phosphate (79.75%) and purines (18.64%). We found a low occurrence of "infection" stones. The sex ratio was related to stone composition and differed according to the main component. For instance, calcium oxalate dihydrate (COD) was more frequent in men than in women, with a sex ratio of 4.97 versus 2.57 for calcium oxalate monohydrate (COM). On the contrary, calcium phosphate was more frequent in female patients (sex ratio 0.72 versus overall ratio). The high frequency of COD calculi (23.17%) suggests that hypercalciuria is particularly frequent in French patients susceptible to stone formation. For each main component, a specific profile was observed in relation to the sex and age of the patients with stones.

Key words Epidemiology · Urinary stones · Infrared analysis · Sex- and age-related composition · False calculi · Hypercalciuria

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Urolithiasis is a relatively common condition in industrialized countries and affects from 4 to 12% of the population [1, 2, 17, 23, 35]. The prevalence of urolithiasis in France, extrapolated from an epidemiological study performed in one French region is estimated to be about 4% [33]. Not only is urinary stone disease common, but the incidence has increased over several decades in countries with high socioeconomic standards [18, 28]. This trend is related to the increase in calcium oxalate calculi, attributed to the increased consumption of animal proteins [26, 27, 32]. Hitherto, no studies have been reported on the composition of calculi that occur in France. We present the results from 10 617 calculi which were analyzed by infrared (IR) spectrophotometry during the period 1977–1993.

Materials and methods

We analyzed 10 617 calculi from adult patients collected from all parts of France during the period 1977–1993. The structure of each calculus was studied using a stereomicroscope with the object of defining the morphology of the stone [6] and to select representative parts of the stone (nucleus, internal section, and external surface) to determine the molecular and crystalline composition by IR spectroscopy. The proportion of each component was obtained by IR analysis on the powder of the whole stone [3]. The various compounds were identified by comparison with a data base of spectra including either published spectra or spectra of products of synthesis [7–9, 11, 14]. In some cases, it was necessary to complete the compound identification using specific analytical methods such as electrophoresis, chromatography, and immunological and chemical reactions. Both the chemical and the crystalline composition of the stones are reported. The presence of each constituent was numbered when it occurred. Proteins were only considered when located in

Table 1 Presence of components in true urinary calculi (*TM*, triamterene metabolites; *GM*, glafenine metabolites; *NASD*, *N*-acetylsulfadiazine; *NASM*, *N*-acetylsulfamethoxazole; *UA*, uric acid)

		Women		Men		Total	
		n	%	*n*	%	*n*	%
Number of calculi		3194		7244		10438	
Oxalates		2581	80.81	6446	88.98	9027	86.48
	Whewellite	2482	77.71	6223	85.91	8705	83.40
	Weddellite	1484	46.46	4395	60.67	5879	56.32
	Ca-oxalate trihydrate	1	0.03	5	0.07	6	0.06
Phosphates		2683	84.00	5641	77.87	8324	79.75
	Carbapatite	2670	83.59	5617	77.54	8287	79.39
	Struvite	582	18.22	350	4.83	932	8.93
	Amorphous Ca-phosphate	428	13.40	333	4.60	761	7.29
	Whitlockite	238	7.45	294	4.06	532	5.10
	Brushite	52	1.63	124	1.71	176	1.69
	Octo-Ca-phosphate	39	1.22	73	1.01	112	1.07
	Tri-Mg-phosphate	5	0.16	0	0.00	5	0.05
	Newberyite	4	0.13	1	0.01	5	0.05
Carbonates calcite		10	0.31	16	0.22	26	0.25
Purines		506	15.84	1440	19.88	1946	18.64
	Uric acid anhydrous	252	7.89	1051	14.51	1303	12.48
	Uric acid dihydrate	126	3.94	546	7.54	672	6.44
	Ammonium urate	230	7.20	275	3.80	605	4.84
	Sodium urate	15	0.47	62	0.86	77	0.74
	Aluminium-Mg-urate	2	0.06	10	0.14	12	0.11
	Other urates	2	0.06	9	0.12	11	0.11
	2,8-Dihydroxyadenine	3	0.09	6	0.08	9	0.09
	UA monohydrate/amorphous UA	3	0.09	6	0.08	9	0.09
	Xanthine	1	0.03	1	0.01	2	0.02
Cystine		64	2.00	63	0.87	127	1.22
Proteins							
	Proteins	1426	44.65	2412	33.30	3838	36.77
	Mucopolysaccharides	51	1.60	78	1.08	129	1.24
Varying nature							
	Blood/porphyrine (as nucleus)	9	0.28	8	0.11	17	0.16
	Urea	5	0.16	8	0.11	13	0.12
	Ca-citrate	0	0.00	2	0.03	2	0.02
	Ca-bilirubinate	1	0.03	0	0.00	1	0.01
Lipids							
	Triglycerides	81	2.54	61	0.84	142	1.36
	Other lipids[a]	17	0.53	7	0.10	24	0.23
Foreign body ligatures/hair/compress		9	0.28	25	0.35	34	0.33
Drugs							
	TM	14	0.44	17	0.23	31	0.30
	GM	25	0.78	6	0.08	31	0.30
	NASD	3	0.09	17	0.23	20	0.19
	Phenazopyridine	1	0.03	4	0.06	5	0.05
	Opaline silica	0	0.00	4	0.06	4	0.04
	Oxolinic acid	2	0.06	0	0.00	2	0.02
	Flumequine	0	0.00	1	0.01	1	0.01
	Penicillin G	0	0.00	1	0.01	1	0.01
	NASM	0	0.00	1	0.01	1	0.01
	Methylene blue	1	0.03	0	0.00	1	0.01
	Sosim thiopental	1	0.03	0	0.00	1	0.01
	Oxypurinol	0	0.00	1	0.01	1	0.01
	Benzocoumarine (metabolites)	1	0.03	0	0.00	1	0.01

[a] Other lipids: cholesterol, free fatty acid, calcium palmitate

Randall's plaques or in a peculiar area of stone, excluding evenly distributed proteins of the stone matrix. The composition of pure and mixed calculi was also reported according to the main component, i.e., the one having the greatest proportion in the whole stone. On the other hand, pure calculi, defined as calculi containing only one species of crystal with less than 3% matrix, were considered apart. For statistical treatment of results we used the chi-square test.

Results

There were 3316 calculi obtained from women and 7301 from men, giving a male to female sex ratio of 2.20:1. We observed more than 70 different components. Among the 10617 calculi, 179 were shown to be false calculi or artifacts: 122 (3.7%) from women and 57 (0.78%) from men. Excluding false calculi, the sex ratio rose to 2.27:1 and the number of detected constituents was more than 50.

Table 1 gives the complete list of constituents identified in the true calculi. In decreasing order, the most frequent constituents, detected in more than 500 calculi, were calcium oxalate monohydrate (COM), carbapatite (CA), calcium oxalate dihydrate (COD), proteins (mainly albumin), anhydrous uric acid (UA0), ammonium magnesium phosphate (struvite, MAP), amorphous calcium phosphate (ACP), uric acid dihydrate (UA2), whitlockite (WK), and hydrogen ammonium urate (AmU). Proteins included especially albumin, fibrin, beta-2-microglobulin, lysozyme, and alpha-1-microglobulin. Ligatures included catgut, polyglycolic acid, flax, polyamide-6, polyester, and suture clips. The following drugs were found: glafenine metabolites (GM) included glafenic acid mixed with hydroxy-2-glafenic acid and hydroxy-4′-glafenic acid; triamterene metabolites (TM) included hydroxy-4′-triamterene sulfate mixed with various proportions of triamterene, hydroxy-4′-triamterene, and other metabolites; *N*-acetylsulfadiazine alone or mixed with sulfadiazine (NASD), and *N*-acetylsulfamethoxazole chlorhydrate (NASM).

Table 2 Frequencies of components in calculi (*COM*, calcium oxalate monohydrate; *COD*, calcium oxalate dihydrate; *CA*, carbapatite; *BR*, brushite; *WK*, whitlockite; *ACP*, amorphous calcium phosphate; *MAP*, ammonium magnesium phosphate; *UA0*, anhydrous uric acid; *UA2*, uric acid dihydrate; *AmU*, hydrogen ammonium urate; *NaU*, sodium urate; *AlU*, aluminium magnesium urate; *DHAd*, 2-8-dihydroxyadenine; *MPS*, mucopolysaccharides)

		Pure						Main components (pure and mixed)							
		Women		Men		Total		Women		Men		Total		Sex ratio (M/F)	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%		
True calculi		235	100	483	100	718	100	3194	100	7244	100	10438	100	2.27	*P*[a]
Oxalates															
	COM	118	50.21	256	53.00	374	52.09	1250	39.14	3218	44.42	4468	42.81	2.57	*
	COD	3	1.28	21	4.35	24	3.34	405	12.68	2013	27.79	2418	23.17	4.97	****
Phosphates															
	CA	18	7.66	13	2.69	31	4.32	941	29.46	658	9.08	1599	15.32	0.70	****
	BR	0	0.00	2	0.41	2	0.28	30	0.94	75	1.04	105	1.01	2.50	*NS*
	WK	0	0.00	0	0.00	0	0.00	11	0.34	10	0.14	21	0.20	0.91	
	ACP	0	0.00	0	0.00	0	0.00	6	0.19	4	0.06	10	0.10	0.67	
	MAP	0	0.00	0	0.00	0	0.00	159	4.98	132	1.82	291	2.79	0.83	****
Carbonates calcite		0	0.00	0	0.00	0	0.00	2	0.06	2	0.03	4	0.04	1.00	
Purines															
	UA0	25	10.64	91	18.84	116	16.16	166	5.20	752	10.38	918	8.79	4.53	****
	UA2	1	0.43	10	2.07	11	1.53	22	0.69	112	1.55	134	1.28	5.09	**
	AmU	3	1.28	2	0.41	5	0.70	33	1.03	24	0.33	57	0.55	0.73	**
	NaU	1	0.43	2	0.41	3	0.42	2	0.06	9	0.12	11	0.11	4.50	
	AlU	0	0.00	1	0.21	1	0.14	1	0.03	3	0.04	4	0.04	3.00	
	DHAd	3	1.28	5	1.04	8	1.11	3	0.09	6	0.08	9	0.09	2.00	
	Xanthine	1	0.43	0	0.00	1	0.14	1	0.03	1	0.01	2	0.02	1.00	
Cystine		42	17.87	33	6.83	75	10.45	63	1.97	61	0.84	124	1.19	0.97	***
Proteins		7	2.98	27	5.59	34	4.74	55	1.72	113	1.56	168	1.61	2.05	*NS*
MPS		7	2.98	6	1.24	13	1.81	18	0.56	19	0.26	37	0.35	1.06	
Blood		2	0.85	3	0.62	5	0.70	6	0.19	4	0.06	10	0.10	0.67	
Drugs		4	1.70	11	2.28	15	2.09	19	0.59	27	0.37	46	0.44	1.42	*NS*
Foreign body		0	0.00	0	0.00	0	0.00	1	0.03	1	0.01	2	0.02	1.00	

[a] Comparison of sex ratios with the overall sex ratio (2.27): *NS*, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

The frequency with which main components were found to occur in this series of 10 438 stones is shown in Table 2. Stones with a single crystalline component mainly comprised COM (52.09%), UA0 (16.16%), and cystine (10.45%). Four crystalline species, namely whewellite (COM), weddellite (COD), carbapatite, and anhydrous uric acid occurred as the main component in more than 5% of calculi. The overall male to female sex ratio was high (2.27); it varied with the crystalline composition of calculi. Calcium oxalate lithiasis was preponderant in both sexes. The proportion of COM lithiasis was slightly higher in men than in women. Lithiasis mainly composed of COD was more frequent in men, as shown by a sex ratio of 4.97. In contrast, phosphate stones were most frequent in women (0.70), except for brushite stones, which were similar in both men and women. The frequency of cystine calculi was twice as high in women as in men (sex ratio 0.97), whilst the opposite was true for uric acid and urate calculi, where the male to female sex ratio was more than 4.5.

Among the 10 438 calculi, 718 (6.9%) stones consisted of only one, 2360 (22.6%) of two, 5527 (52.9%) of three, 1427 (13.7%) of four, and 406 (3.9%) of more than four components. The most frequent associations found in our series were COM-COD-CA (29.7%) and COM-COD-CA-proteins (10.8%).

The age distribution of patients at the time the stone was removed or spontaneously expelled is shown in Fig. 1 for calcium phosphate and uric acid stones, according to sex and type of main component. Calcium phosphate stones (Fig. 1A) were found more often in young women than in older women (30.8% in the range 30–39 years versus 7.0% in the range 60–69 years, $P < 0.0001$). For male patients the difference was not significant. In contrast, the age distribution of patients with "infection" stones (not shown) such as ammonium magnesium phosphate stones showed a significant peak in each population: at an early age, 27.7% in the range 30–39 years versus 5.4% in the range 60–69 years for women ($P < 0.02$) and at an older age, 25.2% in the range 60–69 years versus 7.2% in the range 40–49 years for men ($P < 0.00001$). Uric acid stones (Fig. 1B) were found at an older age in men and women, respectively, 27.4% and 25.6% in the range 60–69 years versus 5.2% and 4.4% in the range 30–39 years ($P < 0.00001$), with a high sex ratio of 4.53. At last, in both sexes, COM and COD calculi were observed in the middle age range 30–60 years, although COD were more frequent in men in the age range 30–39 years (30.8%) than in the range 40–49 years (22.8%; $P < 0.001$). The occurrence of COD as main component decreased after 49 years in both sexes, but, after the age of 60, significantly faster in men than in women.

Figure 2 shows the increase in the overall sex ratio during the period 1981–1993.

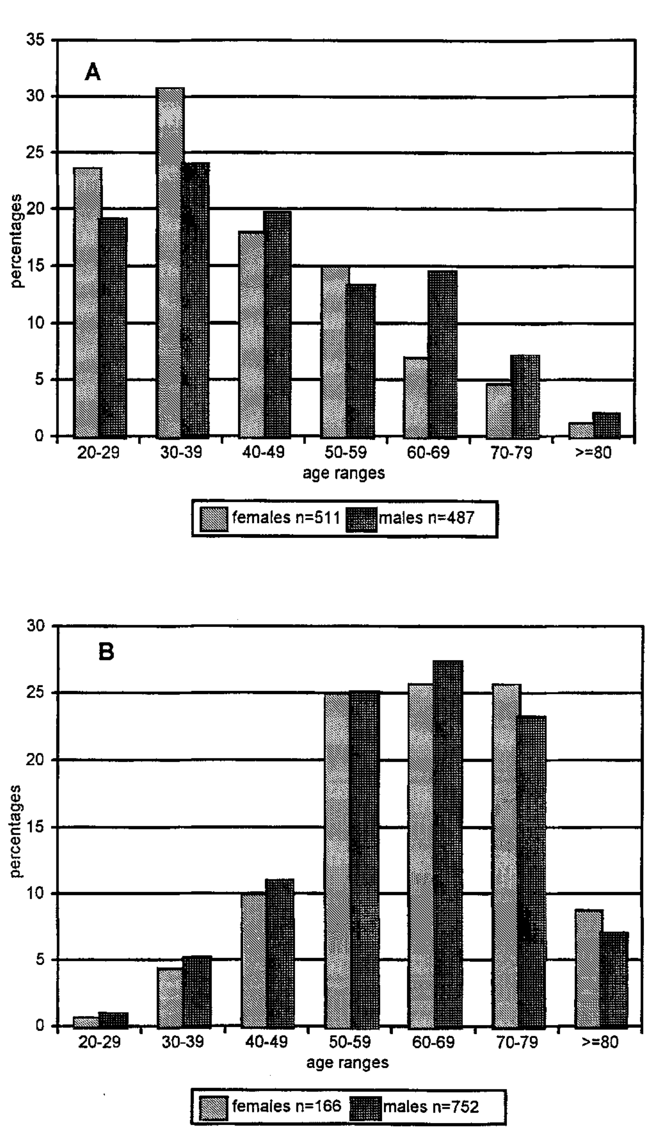

Fig. 1 **A** Age distribution of patients with calcium phosphate calculi (carbapatite, brushite) and free from struvite. **B** Age distribution of patients with uric acid calculi

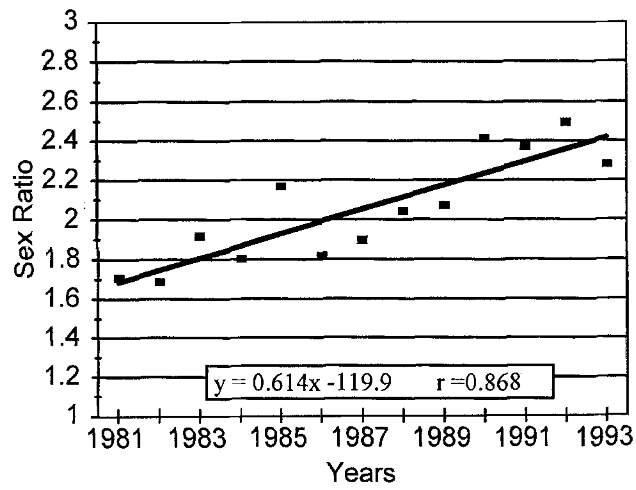

Fig. 2 Evolution of sex ratio (men–women) among patients with calculi for the period 1981–1993

Discussion

In agreement with large series already published [4, 13, 15, 22, 31], calcium oxalate was the most frequent component of urinary calculi and also the most frequent main component observed in two-thirds of stones in this study (Table 3). Pure calcium oxalate stones were found in only 3.81% of cases (Table 4), in contrast with other studies reporting much higher occurrences, from 12.37% for Herring [13] to 25.53% for Brien et al. [4]. Two explanations for such discrepancies can be found in the methods used for stone analysis. First, as reported by Brien et al. [4] and by Leusmann et al. [22], the poor sensitivity of X-ray diffraction to detect minor proportions of apatite is responsible for underestimation of its occurrence in calculi. Secondly, we selectively analyzed by microsampling all significant areas, including nucleus, inner layers, and surface, so that we were able to detect small quantities of compounds such as apatite or proteins, indiscernible by current methods, in the powder of the whole stone. As a consequence, we observed only 22.6% of double association and 52.9% of triple association, while other authors who applied X-ray diffraction [4, 15] found more than 50% of double association and less than 20% of triple association. Using a combination of X-ray diffraction and scanning electron microscopy (SEM), Leusmann et al. [22] found intermediate results. They showed that the combination of the two methods allowed a higher detection rate of apatite, struvite, whitlockite, and ammonium urate than the X-ray diffraction method alone. In fact, when compared with a series of calculi analyzed by IR spectroscopy, our results remain lower in terms of frequency of pure calculi because of the detection of minor components: 6.15% versus 37.0% in data published by Koide (Table 4). Indeed, the high sensitivity of our methodology allowed us, for instance, to detect carbapatite in 79.40% of calculi versus 67.2% in data published by Koide [19]. Because of traces of apatite frequently detected in calcium oxalate stones in our series, we found the smallest frequency of pure calcium oxalate stones. In addition we frequently observed proteins poorly detected by IR spectroscopy in macrosamples and never detected by X-ray diffraction.

Comparing our data with those already published, expressed as frequencies of main components (Table 3), we found similar results but some discrepancies. For instance, although all authors agree on the preponderance of calcium oxalate lithiasis, they disagree about the relative frequency and the sex ratio of COD calculi. Such stones are known to be dependent on hypercalciuria [5, 6, 10, 24], itself closely related to calcium intake [16, 20]. Indeed, when biological data were available, hypercalciuria was found in 78.2% of patients with COD calculi (72% in women and 80% in men). Some hypotheses may be suggested to explain the high occurrence of COD as main component in calculi in our series: (1) the periods observed were different, so the risk factors may have changed; and (2) the populations were also different and the dietary habits were probably not the same, as suggested by the lowest European COM/COD ratio values (Table 3). This observation is suggestive of a higher occurrence of hypercalciuria in French calcium oxalate stone formers. To support this finding, Simon et al. reported hypercalciuria in more than 60% of calcium stone formers in France [33], when Tiselius et al. found hypercalciuria in only 40% of stone formers in Sweden [34]. On the other hand, we observed the highest sex ratio for COD calculi (4.97 versus 2.27), suggesting that the hypercalciuric state is more frequent in French male stone formers.

As did Leusmann [22], we found a relatively low frequency of infection lithiasis compared with the early series (Table 3). This is probably due, first, to earlier detection of urinary infections and, secondly, the greater attention paid to their treatment in recent years. This is confirmed by the progressive decrease in the frequency of infection stones, as shown in the chronological data presented in the table. In contrast, we and Leusman found a high frequency of stones with calcium phosphate as the main component compared with other authors. This might be due to the exposure of metabolic disturbances which were previously hidden by struvite, a consequence of urinary tract infection induced by urea-splitting bacteria. The frequencies of uric acid and cystine stones were similar to those of the other series.

The relationship between frequency of main types of stones and patient age was approximately the same as reported by Robertson [29]. We noted, however, that COD calculi occurred earlier in male than in female patients and decreased more quickly with age. The smaller decrease in COD calculi in women could be related to menopause, with demineralization and consequently hypercalciuria. As already observed by Robertson, the peak of frequency of MAP calculi in women coincided with the child-bearing period and might be related to possible urinary stasis during pregnancy. The peak of frequency of MAP stones in men was observed in the elderly, when the occurrence of prostatic hyperplasia is high. The frequency of uric acid calculi increased with patient age in both sexes, but in male patients was more frequent. The cause is still poorly understood. Actually, the significance of the changes observed in age distribution of the various main components should be moderated by comparison with the age distribution of patients in the healthy population of both sexes.

The sex ratio (men to women) changed with the crystalline composition of calculi. In agreement with Leusmann [21] and Koide [19], the overall sex ratio in our study slowly increased during the period 1981–1993 (1.71–2.28), but this finding is in contradiction

Table 3 Comparison of the frequencies of main components with the published data of other authors (*A*, apatite; other abbreviations as in Table 2)

		Herring 1962[a] [13]: 10 000 calculi		Hesse 1976 [15]: 17 313 calculi		Schneider 1981 [31]: 100 000 calculi		Brien 1982 [4]: 10 000 calculi		Leusmann 1990[a] [22]: 5035 calculi		This study: 10 438 calculi	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Oxalates		7230	73.07	11 166	64.49	69 405	69.41	7180	71.80	2289	45.46	6686	65.97
	COM	3136	31.69	8253	47.67	56 067	56.07	5739	57.39	1837	36.48	4468	42.81
	COD	4094	41.37	2763	15.96	13 338	13.34	1441	14.41	452	8.98	2418	23.17
Phosphates		1730	17.48	2156	12.45	9901	9.90	1401	14.01	1270	25.22	2030	19.45
	MAP	912	9.22	1214	7.01	4906	4.91	525	5.25	105	2.09	291	2.79
	BR	131	1.32	43	0.25	243	0.24	35	0.35	62	1.23	105	1.02
	A or CA	672	6.79	886	5.12	4752	4.75	835	8.35	1098	21.81	1599	15.32
Uric acids		740	7.48	2642	15.26	13 574	13.57	1144	11.44	367	7.29	1052	10.08
	UA0			2102	12.14	10 997	11.00	874	8.74	315	6.26	918	8.79
	UA2			540	3.12	2 577	2.58	270	2.70	52	1.03	134	1.28
	Urates	15	0.15	99	0.57	372	0.37	40	0.40	9	0.18	72	0.69
	NaU	4	0.04	6	0.03			7	0.07	1	0.02	11	0.11
	AmU	8	0.08	93	0.54	372	0.37	33	0.33	8	0.16	57	0.55
	DHAd											9	0.09
	Xanthine	1	0.01	1	0.01			0	0.00			2	0.02
Cystine		88	0.89	65	0.38	181	0.18	26	0.26	43	0.85	124	1.19
Proteins		59	0.60	164	0.95					58	1.15	168	1.61
Blood		26	0.26									10	0.10
MPS		3	0.03									37	0.35
Drugs		3	0.03									46	0.44
Foreign body												2	0.02
Total		9895	99	16 293	94	93 433	93	9791	98	4036	80	10 438	100

[a] Main component >50%

Table 4 Comparison of pure calculi frequencies with the published data of other authors (abbreviations as in Table 2)

		Herring 1962 [13][a] (*n* = 10000)		Brien 1982 [4][a,b] (*n* = 10000)		Koide 1986 [19][c] (*n* = 2724)		This study[c,d] (*n* = 10438)	
		n	%	*n*	%	*n*	%	*n*	%
Oxalates		1237	12.37	2553	25.53	672	24.70	398	3.81
	COM	690	6.90	2494	24.94			374	3.58
	COD	547	5.47	59	0.59			24	0.23
Uric acids		350	3.50	265	2.65	110	4.00	127	1.22
	UA0			235	2.35			116	1.11
	UA2			30	0.30			11	0.11
	Urates	3	0.03	10	0.10	11	0.40	9	0.09
	AmU	3	0.03	6	0.06			5	0.05
	NaU	0	0.00	4	0.04			3	0.03
Phosphates		58	0.58	144	1.44	184	6.75	33	0.32
	BR	17	0.17	5	0.05	10	0.40	2	0.02
	MAP	12	0.12	32	0.32	174	6.40	0	0.00
	A or CA	29	0.29	107	1.07			31	0.30
Cystine		65	0.65	24	0.24	31	1.10	75	0.72
Total		1713	17.13	2996	29.96	1008	37.00	642	6.15

[a] X-ray diffraction
[b] Polarizing microscopy
[c] IR spectroscopy
[d] Optical microscopy

with the assumptions of the literature maintaining that the sex ratio tends toward 1 [2, 30, 35].

Based on large analytical series in the literature, factitious urolithiasis could concern about 1% of calculi: 0.8% of a series of 25000 calculi for Prien [25] and 0.85% of a series of 10000 calculi for Herring [13]. Among 10617 stones, we found 179 (1.6%) spurious calculi. This frequency is intermediate between the values reported previously and those published by Gault et al. [12] in a series of 3300 calculi. Among these factitious calculi, 65% were accidentally submitted to analysis by patients or medical staff, but 35% came from patients who were expecting psychoaffective assistance from physicians.

Conclusion

The combination of two analytical methods, namely optical examination and selective IR spectroscopy involving microsamples of all the significant areas of stones, appears to be a good procedure for identification of urinary stones. Our results emphasize the low frequency of infection stones and, in contrast, the high frequency of COD calculi, suggesting that the hypercalciuric state is a common finding in French stone formers. The analytical composition of stones is essential in the study of the genesis and the treatment of urolithiasis. Other epidemiological aspects of urolithiasis would be of interest for urologists, in particular the localization and the ease of expulsion of the stones in relation to their composition.

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