

Hematoidin crystals in sputum smears: Cytopathology and clinical associations

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Abstract:

BACKGROUND: There has been limited correlation of hematoidin crystals (HC) in sputum with clinical and diagnostic characteristics, partly because they are difficult to recognize. The aim of this study was, therefore, to assess these relationships in a consecutive cohort of sputum samples from patients submitted to a cytology laboratory.

METHODS: Adequate sputum samples from 489 individuals were enrolled in this study. These were fixed in ethanol, stained by the Papanicolaou method and examined microscopically. The normality of the distribution of the continuous variable (age in years) was examined using the Shapiro–Wilk normality test. As the data were not normally distributed, Kendall rank correlation was used to correlate age with the presence of HC. The Pearson’s Chi-square test was used to determine if the proportion of cases with the presence of HC was different among the categorical data variables. A univariate binary logistic regression was used to determine the variables most strongly associated with HC presence. The results include odds ratios, 95% confidence intervals, Wald χ^2 statistics, and corresponding *P* values, with statistical significance assumed at *P* ≤ 0.05. Analyses were performed using IBM SPSS Statistics 22.

RESULTS: The presence of HC in sputum was associated with increasing age (*P* < 0.01), current smoking (*P* < 0.001), chronic occupational exposure to dust (*P* = 0.001), and hemoptysis (*P* < 0.001). These crystals were most prevalent in patients with a diagnosis of carcinoma (93.9%), bronchiectasis (48%), silicosis (16.0%), and chronic obstructive pulmonary disease (0.8%). Crystals were not found in patients with acute bronchitis, asthma, or lung infections, including viral and bacterial pneumonia.

CONCLUSIONS: There are clear associations linked to the finding of HC in sputum that may be helpful in pointing to a specific diagnosis, such as the possibility of underlying carcinoma.

Keywords:

Carcinoma, clinical associations, cytology, hematoidin crystals, lung, Papanicolaou method, sputum smears

Hematoidin (from the Greek word “*haima*” meaning blood and “*eidōs*” meaning resemblance) is a type of endogenous pigment derived from hemoglobin (specifically from protoporphyrin IX) that forms under conditions with low or no oxygen concentration.^[1] Unlike hemosiderin, these crystals do not contain iron, and as such, they are not highlighted by stains such as Perls and Prussian blue. Hematoidin is observed with light microscopy as refractive crystals, and under polarized light, it may have positive or negative birefringence.

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Hematoidin crystals (HCs) may be intra or extracellular; may display a range of morphology, most frequently, a star like or rhomboidal/rectangular shape arranged in a radial pattern; and/or manifest as individual needles that measure 8–10 μm in size and exhibit golden, brown-yellow, or yellowish reddish coloration. HCs are soluble in chloroform but insoluble in water, alcohol, and ether. They were first described in hemorrhagic tissues by Rudolph Virchow in 1847.^[2] Later, Fisher (1923) and Richi (1925) demonstrated the close similarity between hematoidin and bilirubin using a range of chemical methods.^[3,4]

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HCs are often found in body cavities containing old blood and organizing hematomas.^[5] When red blood cell extravasation occurs in closed tissue compartments, hematoidin forms as a result of anaerobic breakdown of the hemoglobin under low oxygen tension. This takes approximately 2 weeks after a bleed, so its finding may help to evaluate the timeframe of bleeding in forensic histopathology.^[6] Following red blood cell degeneration, porphyrin is released from hemoglobin and converted to biliverdin, which is reduced to HCs. Because hematoidin can be converted back to biliverdin, it is not always visible and hence, it is often not detected in biological samples.^[7,8]

There are a number of reports of the presence of HCs in cytopathology, mainly in cervicovaginal smears from pregnant women or pregnancy-like states.^[9-14] In such cases, HCs are also known as “cockleburrs.”^[15] References to these crystals in articles discussing other types of cytological samples, such as those from the respiratory tract, are scarce.^[16-18]

The aim of this study was to examine conventional sputum smears for the presence of HCs, to determine their prevalence and to assess their relationship with demographic and clinical characteristics, in a set of pulmonary pathology samples submitted to a cytology laboratory.

Methods

A retrospective 3-year cohort study (January 2016 to December 2018) was undertaken of samples from 535 consecutive patients, who provided 1219 samples as a result of spontaneous expectorations (2392 sputum smears). All the samples were submitted to the INCLÍNICA Foundation laboratory. Individual-level consent was not sought, as these data are routinely collected by the first author’s laboratory and all analyses were undertaken using fully anonymized data. The study was undertaken in compliance with the principles laid out in the Declaration of Helsinki.

The samples were assessed using a “pick-up-smear” technique. A small quantity of expectorate was selected and placed on a glass slide. With the help of another glass slide, a smear was made by displacing the glass slides in opposite directions. The smears were fixed in 96% ethanol, stained by the Papanicolaou method, and examined for the presence of HCs. The following variables were extracted from the medical records of these patients: age, gender, smoking status, working in an environment affected by dust pollution (e.g., coal mining or metallurgic industry), and the presence or absence of hemoptysis. Clinical and histopathological diagnoses were also recorded, based on information recorded in the medical records.

Statistical analysis

The normality of the distribution of the continuous variable (age in years) was examined using the Shapiro–Wilk normality test. As the data were not normally distributed, Kendall rank correlation was used to correlate age with the presence of HC. The Pearson’s Chi-square test was used to determine if the proportion of cases with the presence of HC was different among the categorical data variables (gender, smoking history, occupational dust exposure, hemoptysis, and diagnostic categories). A univariate binary logistic regression was used to determine the variables most strongly associated with HC presence. The results include odds ratios, 95% confidence intervals, Wald χ^2 statistics, and corresponding *P* values, with statistical significance assumed at $P \leq 0.05$. Collinearity was ruled out using a tolerance >0.2 and a variance inflation factor <5 . Once multicollinearity was ruled out, a multiple binary logistic regression analysis was used, with the variables identified in the univariate analysis as having the strongest relationship with HC. Analyses were performed using IBM SPSS Statistics 22 (SPSS Inc. Chicago, Illinois. USA).

Results

A total of 489 of the initial cohort of 535 consecutive patients were included in the study (46 patients were excluded because their samples were classified as not valid or insufficient due to the absence of alveolar macrophages and abundance of pavementous cells, indicating that the samples were primarily made up of saliva). This excluded 92 sputum smears. Of the remaining 2300 sputum smears, 5.95% (137 smears) contained HCs, corresponding to 13.09% of the cohort of patients (64 patients).

A descriptive summary of patient age and presence of HC is shown in Table 1. The results of the correlation analysis demonstrate that sputum samples from older patients were more likely to have HCs present ($r_t = 0.191$, $P < 0.001$).

Table 2 shows the proportion of HC presence among the categorical variables. Most of the patients were male (92% overall), current smokers (65% overall), do not have occupational dust exposure (84% overall), did not have hemoptysis (59% overall), and had chronic

Table 1: Age and hematoidin crystals’ presence

Age (years)	No HC present	HC present
Minimum	34	56
Maximum	76	76
Median	60	66
Average	61.0	65.5

HC=Hematoidin crystals

Table 2: Presence of hematoidin crystals by gender, smoking history, occupational dust exposure, hemoptysis, and diagnostic category

	No HC present		HC present		Total	
	n	Percentage (out of n=425)	n	Percentage (out of n=64)	n	Percentage (out of n=489)
Gender						
Male	393	92	57	89	450	92
Female	32	8	7	11	39	8
Smoking history						
Nonsmoker	71	17	1	2	72	15
Former smoker	94	22	7	11	101	21
Current smoker	260	61	56	88	316	65
Occupational dust exposure						
No	346	81	63	98	409	84
Yes	79	19	1	2	80	16
Hemoptysis						
No	287	68	0	0	287	59
Yes	138	32	64	100	202	41
Diagnosis						
Acute bronchitis	35	8	0	0	35	7
Asthma	20	5	0	0	20	4
Bacterial pneumonia	3	1	0	0	3	1
Bronchiectasis	13	3	12	19	25	5
COPD	252	59	2	3	254	52
Cough	42	10	0	0	42	9
Pulmonary fibrosis	12	3	0	0	12	2
Silicosis	21	5	4	6	25	5
Tuberculosis	2	0	0	0	2	0
Viral pneumonia	22	5	0	0	22	4
Carcinoma						
Adenocarcinoma	3	1	14	22	17	3
Small-cell lung carcinoma	0	0	8	13	8	2
Squamous cell carcinoma	0	0	24	38	24	5
Total	425	-	64	-	489	-

HC=Hematoidin crystals, COPD=Chronic obstructive pulmonary disease

obstructive pulmonary disease (COPD) (52% overall). Compared to cases with no HC presence, sputum cases that had HC present had a higher proportion of: smokers (88% vs. 61%); hemoptysis (100% vs. 32%); bronchiectasis (19% vs. 3%); adenocarcinoma (22% vs. 1%); small cell lung carcinoma (13% vs. 0%); and squamous cell carcinoma (38% vs. 0%).

Compared to cases with no HC present, sputum cases that had HC present had a lower proportion of: males (89% vs. 92%); nonsmokers (2% vs. 17%); former smokers (11% vs. 22%); occupational dust exposure (2% vs. 19%); acute bronchitis (0% vs. 8%); asthma (0% vs. 5%); COPD (3% vs. 59%); cough (0% vs. 10%); pulmonary fibrosis (0% vs. 3%); and viral pneumonia (0% vs. 5%). The proportion of HC absent and present was similar (within 1% point) in cases with diagnoses of bacterial pneumonia, silicosis, and tuberculosis.

Table 3 shows the results of the Pearson's Chi-square test, which identifies statistically significant associations between the presence or absence of HC

and gender, smoking history, occupational dust exposure, hemoptysis, and diagnosis. Dichotomous variables (gender, occupational dust exposure, and hemoptysis) had the same statistical results for each variable.

There was a statistically significant relationship between HC presence and several of the variables in Table 3. Current smokers were more likely to have HC present than absent ($\chi^2 = 16.859$, $P < 0.001$). Conversely, nonsmokers and former smokers were more likely to have no crystals ($\chi^2 = 10.159$, $P = 0.001$; $\chi^2 = 4.242$, $P = 0.039$, respectively). Patients with occupational dust exposure were more likely to have no crystals ($\chi^2 = 11.784$, $P = 0.001$). Diagnoses of acute bronchitis, COPD, and cough were more likely to have no HCs ($\chi^2 = 5.677$, $P = 0.017$; $\chi^2 = 70.303$, $P < 0.001$; $\chi^2 = 6.919$, $P = 0.009$, respectively). Hemoptysis is more likely to be associated with HCs present ($\chi^2 = 104.624$, $P < 0.001$). Diagnoses of bronchiectasis, adenocarcinoma, small-cell lung carcinoma, and squamous cell carcinoma were more likely to have HC present than absent ($\chi^2 = 28.231$,

$P < 0.001$; $\chi^2 = 74.283$, $P < 0.001$; $\chi^2 = 54.009$, $P < 0.001$; $\chi^2 = 167.601$, $P < 0.001$, respectively).

There was no significant association between HC present and gender ($\chi^2 = 0.880$, $P = 0.348$), or diagnoses of asthma ($\chi^2 = 3.140$, $P = 0.076$), bacterial pneumonia ($\chi^2 = 0.455$, $P = 0.500$), pulmonary fibrosis ($\chi^2 = 1.853$, $P = 0.173$), silicosis ($\chi^2 = 0.196$, $P = 0.658$), tuberculosis ($\chi^2 = 0.302$, $P = 0.582$), or viral pneumonia ($\chi^2 = 3.469$, $P = 0.063$).

Regression analysis

A univariate logistic regression analysis was performed for HCs present with age (in years), gender, smoking

history, occupational dust exposure, hemoptysis, and diagnosis in order to identify which variables to include in a multiple regression model. The diagnosis variable was coded as cases with carcinoma (including adenocarcinoma, small-cell lung carcinoma, and squamous cell carcinoma) or no carcinoma (all other diagnoses). For dichotomous variables, the absence of the variable (code of 0) was the reference for comparison when applicable (males were used as the reference for gender). For the ordinal variable of smoking history, the reference variable was nonsmoker. Table 4 shows the univariate logistic regression results for the variables included in each univariate regression.

Table 3: Comparison of presence of hematoidin crystals by gender, smoking history, occupational dust exposure, hemoptysis, and diagnostic category

	No HC present, percentage (out of n=425)	HC present, percentage (out of n=64)	Higher proportion for HC presence	χ^2	P
Gender					
Male	92.5	89.1	No	0.880	0.348
Smoking history					
Nonsmoker	16.7	1.6	No	10.159	0.001
Former smoker	22.1	10.9	No	4.242	0.039
Current smoker	61.2	87.5	Yes	16.859	<0.001
Occupational dust exposure					
Yes	18.6	1.6	No	11.784	0.001
Hemoptysis					
Yes	32.5	100.0	Yes	104.624	<0.001
Diagnosis					
Acute bronchitis	8.2	0.0	No	5.677	0.017
Asthma	4.7	0.0	No	3.140	0.076
Bacterial pneumonia	0.7	0.0	No	0.455	0.500
Bronchiectasis	3.1	18.8	Yes	28.231	<0.001
COPD	59.3	3.1	No	70.303	<0.001
Cough	9.9	0.0	No	6.919	0.009
Pulmonary fibrosis	2.8	0.0	No	1.853	0.173
Silicosis	4.9	6.3	Yes	0.196	0.658
Tuberculosis	0.5	0.0	No	0.302	0.582
Viral pneumonia	5.2	0.0	No	3.469	0.063
Carcinoma					
Adenocarcinoma	0.7	21.9	Yes	74.283	<0.001
Small-cell lung carcinoma	0.0	12.5	Yes	54.009	<0.001
Squamous cell carcinoma	0.0	37.5	Yes	167.601	<0.001

HC=Hematoidin crystals, COPD=Chronic obstructive pulmonary disease

Table 4: Binary logistic regression analysis hematoidin crystals' presence with each variable, sorted by Wald χ^2

Predictor	B	Wald χ^2	P	OR Exp(B)	95% CI for Exp(B)	
					Lower	Upper
Diagnosis - carcinoma	5.885	83.849	<0.001	359.481	102.012	1266.777
Age (years)	0.127	23.939	<0.001	1.135	1.079	1.194
Smoking history						
Nonsmoker (B0)		12.960	0.002			
Former smoker (B1)	1.665	2.375	0.123	5.287	0.636	43.953
Current smoker (B2)	2.727	7.181	0.007	15.292	2.081	112.401
Occupational exposure to dust	-2.666	6.892	0.009	0.070	0.009	0.509
Gender	0.411	0.870	0.351	1.508	0.636	3.577
Hemoptysis	20.435	0.000	0.993	749,205,554.9	0.000	

HC=Hematoidin crystals, OR=Odds ratio, CI=Confidence interval

Diagnosis (carcinoma vs. no carcinoma), age, smoking history, and occupational exposure to dust were each significantly associated with the presence of HCs. These variables were tested for multicollinearity to determine if they could be included in the multiple regression model. Multicollinearity was not a factor between the diagnosis, age, smoking history, and occupational exposure [Table 5], and therefore a multiple binary logistical regression was performed with the four independent variables and presence of HCs. The results of the multiple logistical regression are shown in Table 6, with the classification accuracy shown in Table 7.

Approximately 95% of the sample was correctly classified using this logistic model. Because the y-intercept is negative (-35.837), when each predictor is absent (carcinoma, smoker, and occupational dust exposure) or low (age), HCs' presence was less likely. The largest coefficient in the model equation corresponded with diagnosis (7.583), and therefore the presence of carcinoma had the largest impact on the equation. The odds of HCs' presence in the test group (carcinoma) were higher than the reference group (no carcinoma). The next largest coefficient was for smokers (2.301), supporting that smokers have higher odds of HCs being present than the reference group (nonsmokers). However, the coefficient for former smokers was negative (-0.239), which suggests that the odds of HC being present for former smokers is lower than nonsmokers. The coefficient for occupational exposure (0.944) suggests that a patient who is exposed to occupational dust (test group) has higher odds of having HC than those who are not exposed (reference group). In addition, age

also has a positive coefficient, suggesting that the finding of HC in urine increases with age.

Based on this model, if a 60-year-old patient has no carcinoma, is a nonsmoker, and has no occupational exposure to dust, the following equation could be applied:

$$\text{Predicted logit} = -35.837 + (7.583[\text{carcinoma}]) + (0.477[\text{age}]) + (-0.239[\text{former smoker}]) + (2.301[\text{smoker}]) + (0.944[\text{dust}])$$

$$Z = -35.837 + 7.583(0) + 0.477(60) - 0.239(0) + 2.301(0) + 0.944(0) = -7.217.$$

In order to determine the probability of HC presence, the following equation could be applied:

$$\text{Predicted probability} = e^{-7.217} / (1 + e^{-7.217})$$

$$\text{Predicted probability} = 2.718282^{-7.217} / (1 + 2.718282^{-7.217}) = 0.07\%$$

In other words, the patient would have a 0.07% probability of having HC detected in their sputum sample. By changing the diagnosis to carcinoma, the result changes as follows:

$$\text{Predicted logit} = -35.837 + (7.583(\text{carcinoma})) + (0.477(\text{age})) + (-0.239(\text{former smoker})) + (2.301(\text{smoker})) + (0.944(\text{dust}))$$

$$Z = -35.837 + 7.583(1) + 0.477(60) - 0.239(0) + 2.301(0) + 0.944(0) = 0.366$$

In order to determine the probability of HC being present, the following equation could be applied:

$$\text{Predicted probability} = e^{0.366} / (1 + e^{0.366})$$

$$\text{Predicted probability} = 2.718282^{0.366} / (1 + 2.718282^{0.366}) = 59.05\%$$

In other words, the patient would have a 59.05% probability of having HC in their sputum. In another

Table 5: Collinearity statistic of the independent variables for the multiple regression model

	Collinearity statistics	
	Tolerance	VIF
Diagnosis - carcinoma	0.936	1.068
Age (years)	0.640	1.562
Smoking history	0.909	1.100
Occupational exposure to dust	0.648	1.544

VIF=Variance inflation factor

Table 6: Binary logistic multiple regression analysis

Predictor	B	Wald χ^2	P	OR Exp(B)	95% CI for Exp(B)	
					Lower	Upper
Diagnosis - carcinoma	7.583	48.009	<0.001	1965.195	230.039	16788.410
Age (years)	0.477	25.935	<0.001	1.611	1.341	1.935
Smoking history						
Nonsmoker (B0)		13.787	0.001			
Former smoker (B1)	-0.239	0.034	0.854	0.788	0.062	9.945
Current smoker (B2)	2.301	3.481	0.062	9.985	0.890	111.984
Occupational exposure to dust	0.944	0.160	0.689	2.570	0.025	263.423
Constant	-35.837	30.327	<0.001	0.000		

OR=Odds ratio, CI=Confidence interval

Table 7: Classification table

Observed	Predicted			Percentage correct
	No HC present	HC present	Total	
No HC present	420	5	425	98.8
HC present	19	45	64	70.3
Total	439	50	489	95.1

HC=Hematoidin crystals

example, if a 57-year-old patient has carcinoma, is a current smoker, and has occupational exposure to dust, the following equation could be applied:

$$\text{Predicted logit} = -35.837 + (7.583(\text{carcinoma})) + (0.477(\text{age})) + (-0.239(\text{former smoker})) + (2.301(\text{smoker})) + (0.944(\text{dust}))$$

$$Z = -35.837 + 7.583(1) + 0.477(57) - 0.239(0) + 2.301(1) + 0.944(1) = 2.18$$

In order to determine the probability of HCs' presence, the following equation could be applied:

$$\text{Predicted probability} = e^{2.18} / (1 + e^{2.18})$$

$$\text{Predicted probability} = 2.718282^{2.18} / (1 + 2.718282^{2.18}) = 89.84\%$$

In other words, the patient would have an 89.84% probability of HCs being observed.

Some examples of HCs in sputum samples are shown in Figure 1a-d. They demonstrate the range of appearances that were observed in this study.

Discussion

Hematoidin is an endogenous hematic pigment derived from bilirubin, and is unlike other endogenous pigments such as hemosiderin, in that it does not contain iron. Using a Giemsa stain, it can be observed with a light microscope as refractile crystals, with a golden-yellow hue. When stained with the Papanicolaou method, these crystals have a brown yellow to reddish hue. The crystals have been previously described as having the following four forms: star-shaped clusters, irregular crystals, aggregates of minute spheroids, and rhomboid crystals.^[19] We observed similar cytomorphology in our series.

There has been some controversy over the nature and origin of HCs, in part, as they are not easily stained. Izutsu *et al.* demonstrated that across 16 histochemical stains, only the PAS stain was positive for HCs, suggesting that these crystals are composed of a protein-carbohydrate complex, rather than bilirubin or iron on their own.^[20] This is supported by the fact that Brenner *et al.* showed significant morphological similarities between asteroid bodies and intracellular

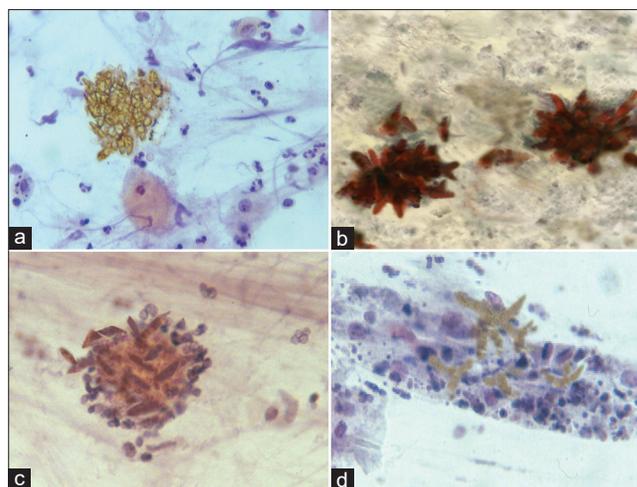


Figure 1: Hematoidin crystals in sputum samples. (a) Cluster of irregular crystals with a golden-yellow hue (Papanicolaou, x400). (b) Two groups of crystals with rhomboidal shape and reddish hue (Papanicolaou, x400). (c) Brownish elongated crystals in a granular background mixed with leukocytes (Papanicolaou, x400). (d) Starfish-shaped crystals with a golden-yellow hue within a background of detritus and macrophages (Papanicolaou, x400)

HCs in three cases of splenic infarcts, providing evidence of lipids as part of the composition of the crystals.^[21] Even so, the origin of HCs as a red cell breakdown product is well attested.^[22,23] HCs have also been found in a range of other types of samples. They have been reported in a cervical abscess,^[24] atypical meningioma,^[25] cerebrospinal fluid,^[26] and in synovial fluid.^[27] However, this study represents one of the largest to examine HCs in association with sputum.

Our research focused on the observation of HCs in a cohort of patients presenting with a range of pulmonary pathologies, where sputum samples were submitted to a cytology laboratory. Pathologies in which chronic bleeding is a recognized feature, such as lung carcinoma,^[28] silicosis, and bronchiectasis, were associated with the presence of HCs. The prevalence of such crystals in patients with COPD was low, and they were not found in the sputa of patients with several other benign lung conditions (e.g., bronchitis, asthma, and pneumonia). Experiments in rats have shown that HCs are not only a manifestation of bleeding, but may also have pro-inflammatory properties and thus are chemotactic to neutrophils.^[29] Indeed, in our series, we frequently observed these crystals in close proximity to inflammatory cells and debris.

In this study, lung carcinoma was the clinical condition most frequently associated with the presence of HCs in sputum smears. HCs were observed in 100% of sputa from patients with squamous cell carcinoma as well as small-cell carcinoma. They were also observed in association with lung adenocarcinoma, but only in 35.2% of these cases. This difference in the prevalence of HCs across the

different types of lung carcinomas is likely due to the fact that bleeding and tissue necrosis are particularly common in squamous and small-cell carcinoma.^[30] In fact, it is because of the increased risk of bleeding in squamous cell carcinoma of the lung that patients often do not receive bevacizumab anti-vascular endothelial growth factor monoclonal antibody therapy.^[31]

In nonmalignant pulmonary conditions, the prevalence of HCs was highest in sputum smears from patients with bronchiectasis. This is in line with the histopathological picture, where chronic bleeding is a common observation in clinical settings such as bronchiectasis, lobar pneumonia, and pulmonary abscess.^[32,33]

There are other types of crystals that may be encountered in lung samples that need to be differentiated from HCs. Charcot-Leyden crystals may be observed in eosinophil-related conditions such as allergic lung diseases, eosinophilic pneumonia, and certain parasitosis.^[34] However, Charcot-Leyden crystals typically form bipyramidal-rhomboid-shaped crystals that resemble a compass needle.^[35] Calcium oxalate crystals are sometimes seen in association with pulmonary *Aspergillus* infection.^[36,37] These are bi-refringent needle-like crystals that typically form rosettes or wheat-sheaf-like arrangements.^[38] Pulmonary crystal-storing histiocytosis, often seen in conjunction with the underlying myeloma or low-grade B-cell lymphoma, to date, has only been reported in a fine-needle aspiration sample.^[39] Exogenous crystals that may be encountered in lung samples include material from aspiration (e.g., talc, barium),^[40] embolization (e.g., total parenteral nutrition),^[41] and drugs (e.g., clofazimine).^[42] Anthracosis and pneumoconiosis-associated crystals (e.g., silicosis) are best identified in tissue specimens.

Conclusions

This study provides evidence that the presence of HCs in sputum samples exhibits strong relationships with demographic and diagnostic variables that may be of value in reaching a correct associated diagnosis, particularly the possibility of an underlying lung carcinoma.

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Conflicts of interest

There are no conflicts of interest.

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