ISOLATED HEMATURIA AS A MORTALITY RISK PREDICTOR

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Introduction

Microscopic evaluation of urine samples looking for cellular elements (including red blood cells [RBCs], white blood cells and casts) has been part of insurance testing since the beginning of the medical evaluation of applicants, similar to how it is used in clinical medicine. However, one possible difference from clinical practice in the collection of insurance urine specimens may be less utilization by applicants of "clean catch" procedures. This lack allows more contamination of urine samples with RBCs and white blood cells from sources other than the urine itself. Because scanning all urines looking for any RBCs is expensive, time consuming and has a low yield, the decision as to which samples undergo microscopy is based on other findings or applicant history.

Beginning in 2001, urine samples handled by Clinical Reference Laboratory (CRL) were routinely screened for the presence of hemoglobin (suggesting the presence of RBCs) as well as leukocyte esterase (an enzyme specific for white blood cells). Also in 2001, manual urine microscopy (which requires centrifuging the urine and review of the sediment by a technician) was replaced by automated flow cytometry, with review of digital images on a screen by the technician as needed and for quality assurance. Flow cytometry does not require centrifugation of the urine sample, so fragile post-collection RBCs were more likely to remain intact. The introduction of flow cytometry resulted in a substantial increase in the percentage of samples found with RBCs. Other indicators to perform flow cytometry at CRL include the presence of leukocyte esterase and for some insurers, the presence of protein or a suggestion of diabetes from testing or history.

Executive Summary *A 6-year follow-up mortality study of 3,719,311 insurance applicants tested both for hematuria and urine hemoglobin found that relatively high levels of urine red blood cells (RBCs) or high levels of urine hemoglobin are associated with excess mortality. This association was absent for women age 20 to 59, most likely due to RBCs from menstruation being common. Further evaluation of hematuria can be safely limited to those samples with high levels of RBCs or hemoglobin, but does not discriminate risk for women age 20 to 59.*

Urine hemoglobin screening is not entirely specific for hemoglobin; it can detect myoglobin from muscle breakdown as a result of very heavy exercise and other substances so that, in practice, a substantial portion of positive urine results for hemoglobin will not be associated with RBCs in the urine. However (especially with insurance urinalysis which is typically delayed for 24 to 96 hours after collection), there can be lysis (disintegration) of urine RBCs so that hemoglobin might be the only remaining evidence of them. In addition, the urine hemoglobin test is more sensitive to free hemoglobin than that which is present in intact RBCs, so low levels of intact RBCs might be insufficient to produce a positive hemoglobin test.

When the presence of urine RBCs is recurrent or continuous, but not severe enough to be visible as a color change, and there are no other findings such as proteinuria, this may be labeled as "isolated microscopic hematuria" (IMH); but the definition regarding the minimum required number of positive urine samples (usually more than one sample) or the required number of RBCs (1 to 5+) is inconsistent.

Various authors have studied isolated persistent or recurrent microscopic hematuria but did not always arrive at the same conclusion, in part because the population studied and definitions of IMH varied. Some have found significant pathology or risk of progressive renal disease.[1,2] Others find less reason for concern.[3,4] Review articles suggest limited risk for IMH but go on to suggest possible work-up or follow-up in some detail.[5-7]

A single episode or short period of isolated microscopic hematuria could be the result of urogenital irritation or inflammation, urinary infection or menstrual blood, as well as being secondary to recent heavy exercise. None of these conditions would be expected to be associated with excess mortality risk.

In a population somewhat similar to life insurance applicants, thin basement membrane disease or no finding after biopsy and other evaluation was the most common finding for isolated but persistent microscopic hematuria.[4] Neither finding appears to be associated with excess mortality risk over time. A (usually) less common finding (the relative frequency to thin basement membrane disease is uncertain and may vary in different populations) is IgA nephropathy (Berger's Disease), which over many years may lead to renal failure. This progression to renal failure is far more common when proteinuria is present, and relatively uncommon with a urine protein/creatinine ratio <0.21 mg/mg. In addition, urologic malignancies are occasionally identified based on isolated microscopic hematuria, most commonly at older ages and in those with a smoking history.

The available studies that use very different populations and are based on different definitions of IMH provide limited guidance for the insurance setting. When a urine finding is presented to an underwriter, it must be either accepted, additional evaluation (specimens) required or rated. This led us to utilize the large CRL database of tested applicants for whom we know the vital status based on the Social Security Death Master File to provide more guidance regarding a finding of urine hemoglobin or the presence of urine RBCs.

How the Study Was Done
In this study examining the mortality associated with urine hematuria and hemoglobin, 3,719,311 insurance applicants age 20 to 89 who were tested from 2001 to 2006 were followed up for mortality in 2010 using the Social Security Death Master File. There were 32,061 deaths, and median follow-up was 6 years (range 0 to 9 years).

Urine samples were screened for the presence of white cell leukocyte esterase or red cell hemoglobin with SciTeck Diagnostics Auto UA Urinary Leukocyte Esterase and Auto UA Hemoglobin test on a Roche Diagnostics Modular Analyzer. If the test for either marker was positive, flow cytometry was performed looking for cellular elements using the Iris Diagnostics iQ200 Sprint. Some samples were reflexed for other causes defined by individual insurers. Cell counts for white blood cells and RBCs were taken from the flow cytometry, and were calibrated by the manufacturer to roughly approximate what might be seen per high power field (HPF) on a microscopy of the sediment.

Excluded from our analysis were applicants who had hemoglobin A1c 6% or greater, fructosamine >2.1 mmol/L (if the hemoglobin A1c was missing), urine protein/creatinine ratio >=.21 mg/mg, or eGFR (Rule equation) <60 ml/min/1.73 m². These exclusions were made for two reasons. The first was because we are interested in the mortality specific to the isolated finding of RBCs, not the mortality associated with proteinuria or reduced renal function which clearly has additional risk and requires evaluation. The second reason was because some insurers reflex to flow cytometry based on diabetic history, suggestive laboratory findings or elevated urine protein. These conditions have excess risk and would exaggerate the urine RBC risk if included in the study group.

Mortality ratios were calculated with Cox regression comparing those with no urine RBCs or negative urine hemoglobin (as reference groups) to those with various levels of urine RBCs or hemoglobin based on our selected 2001 to 2006 study population. Mortality ratios were calculated separately for men and for women; each group was further split into two age bands (20 to 59, 60 to 89). The Cox regression analyses included age and smoking status (presence of cotinine >0.2 mg/dL) as covariates.

What the Study Found
Any discussion of underwriting action based on a laboratory finding must include information on the frequency of the finding(s) as well as associated mortality risk. In Table 1 (page 37), the frequency of both elevated urine hemoglobin and the frequency of finding urine RBCs of 0, 1 to 4, 5 to 9 and 10+ cells per HPF are shown for men and women separately, split by age 20 to 59 and 60 to 89. Age 60 was chosen as a break-point because malignancy is rare below that age, and menstrual bleeding is rare at or above that age. The RBC ranges were chosen because they closely match those in common usage in the industry. Negative hemoglobin is <100 µg/dL, while positive hemoglobin is 100+ µg/dL.

For all men and for women age 20 to 59, positive urine hemoglobin was the most common trigger for flow cytometry. However, for women age 60 to 89, more tests were triggered by leukocyte esterase, suggesting the presence of white blood cells. RBCs were found in some of these specimens as well, most often with counts <10 per HPF.

We looked at urine hemoglobin levels vs. number of RBCs in those samples positive for both. Small numbers of RBCs were more likely to have lower levels of hemoglobin and vice versa, but the correlation was poor overall so that accurately predicting one finding from the other is impossible (data not shown). This is not surprising given the expected variation in the degree of hemolysis between urine specimens and the other causes of false-positive hemoglobin results and other triggers for flow cytometry.

We studied the mortality by age and sex of those without RBCs found (or flow cytometry not performed because all screening was negative) compared to those with RBCs of various levels and also urine hemoglobin positive. For those with RBCs, we excluded samples negative for urine hemoglobin since any other trigger for flow cytometry might in itself be associated with excess risk. These results are in Table 2 (page 37). This analysis indicates that the presence of (or number of) RBCs for women age 20 to 59 is not predictive of mortality risk. For the other three age-sex groups, findings of 10+ RBCs per HPF are predictive of an increased mortality risk in the range of 50 to 90%. Samples with fewer than 10 RBCs are not clearly associated with excess risk for any age-sex group. Although counts of 5 to 9 RBCs in men age 60 to 89 may be associated with excess risk, the 95% confidence intervals are very wide and overlapping with relatively few deaths, so this may be a chance result.

Because urine hemoglobin and urine RBC numbers were not tightly correlated, we studied mortality based on hemoglobin level rather than RBC count for those samples shown to have RBCs to find out which approach was more predictive of risk. We compared the mortality of those positive and negative for hemoglobin, and also split the level of hemoglobin into thirds if positive. Hemoglobin presence or level proved less predictive of risk than RBC count by either approach (data not shown).

We also studied urine hemoglobin when RBCs were absent on flow cytometry. False positives made up some of this subpopulation, but samples with all RBCs lysed may have been present as well. When we simply compared the mortality of positive hemoglo-bin samples to negative hemoglobin samples for this group, we found no excess risk for women age 20 to 59, but approximately a 20% or more increased risk for the other three age-sex groups. When examined by level of hemoglobin as shown in Table 3 (page 38), we found almost all the excess risk was limited to those with the highest levels of hemoglobin, presumably associated with lysed RBCs. Excess risk began at a hemoglobin level of 500 µg/dL or higher, representing 13.4% of hemoglobin positives without RBCs (excluding women 20 to 59), while urine hemoglobin values 1,000 µg/dL or higher had at least 200% risk but represented just 6.1% of hemoglobin positives without RBCs.

What Do the Study Results Contribute to Risk Assessment?

For insurance applicants, repeating urine specimens is both costly and poorly received so that decisions made based on the original specimen become very important. The existing literature is based mainly on patients with repeatedly positive urine specimens, may include specimens positive for protein as well, and was likely collected with the superior "clean catch" method. Basing underwriting decisions on this data may therefore lead to an overestimation of risk. Our approach was to exclude applicants who had other adverse findings, and determine risk assessment using the single specimen that is available.

Based on this approach, we determined that when both urine hemoglobin and urine RBC are positive, the urine RBC count was more predictive of risk, likely because it was more closely associated with the presence and number of RBCs in the fresh specimen.

For all men and for women age 60 to 89 an isolated finding of 10 or more RBCs per HPF (17.3% of positive samples) and for men age 60 to 89 possibly a finding of 5 to 9 RBCs (24.9% of positives for this age-sex group) have increased risks, suggesting the need for further evaluation. It is unlikely that evaluation of <10 RBCs per HPF in men age 20 to 59 or women age 60 to 89 will improve risk discrimination.

For women age 20 to 59, hematuria was much more common and there was no increased risk at any level. For these young women, RBCs from menstrual bleeding and spotting may be the reason for the loss of the RBCs' predictive ability for mortality found among the other age-sex groups. It may be possible to identify high RBC counts among women age 20 to 59 that are not caused by menstruation by use of additional history, but the value or reliability of attempting this identification by history is uncertain. We are doubtful such evaluation will improve risk discrimination regardless of self-reported menstrual history.

In the presence of hemoglobin but with no RBCs found, there is a variable risk. Once again, for women age 20 to 59, no excess risk was found since a high percentage of these hemoglobin positives are likely either false positives or menstrual in origin. For older women and all men there was an increase in risk, but that risk was concentrated in those with higher hemoglobin values (500+ μg/dL) representing just 13.4% of those samples with hemoglobin but without RBCs. Laboratory reporting of the level of urinary hemoglobin (which is not currently done) might be of value in deciding which urine samples might require further evaluation. It is likely the excess risk noted is based on hematuria in the original specimen, with complete lysis of RBCs associated with handling and the delay inherent in transport to insurance laboratories.

Conclusion

The presence of RBCs in the urine at levels of 10+ cells per HPF (by flow cytometry) is associated with a moderate increase in mortality risk for all men and for women age 60 to 89, but not for younger women (likely due to menstrual contamination). Urine RBC levels of 5+ cells per HPF may be associated with some risk in men age 60 to 89. Although the level of RBCs appeared to be a superior risk identification tool compared to urine hemoglobin when both were positive, risk was also present when RBCs were not found but hemoglobin values were in the highest 13.4% of samples positive for hemoglobin (excluding women age 20 to 59). These samples likely had substantial numbers of RBCs which had lysed.

To reduce the further evaluation of urine samples with RBCs in the absence of other findings such as proteinuria, while capturing any excess mortality risk, the following criteria may be useful for insurance specimens:

- No further evaluation of younger women (age 20 to 59) with positive RBCs by flow cytometry or positive urine hemoglobin without additional history or findings.
- For all other age-sex groups, use a relatively high RBC cut-off value of 10+ cells per HPF or a minimum of 5+ for men ages 60 to 89.
- For all other age-sex groups, limit evaluation of urine hemoglobin results to those specimens without RBCs and with a urine hemoglobin of 500 μg/dL or higher.

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Vera F. Dolan, MSPH, FALU, Senior Research Scientist at Clinical Reference Laboratory, is a consultant specializing in underwriting research and product development. At CRL, Vera assists with the analysis and publication of CRL's mortality study data. In her consulting practice, Vera develops risk assessment tools for underwriters, including underwriting manuals, automated risk assessment systems and underwriter training. Vera provides litigation support for misrepresentation and other underwriting issues, as well as life expectancy calculations for use during litigation.

Robert L. Stout, PhD, is President and Director of the Clinical Reference Laboratory based in Lenexa, Kansas. He completed undergraduate studies at California State University (Fullerton) and obtained a PhD in Biological Chemistry from UCLA School of Medicine. Since 1978 he has been directly responsible for introducing many of the new tests and procedures used in risk assessment such as urine and saliva HIV. Dr. Stout has produced nine patents over the last decade.

Michael Fulks, MD, Consulting Medical Director, analyzes, interprets and writes up CRL's mortality study results. Dr. Fulks is a graduate of the University of California at Davis, completing his residency at the University of Wisconsin and practicing for 8 years before joining Allmerica in 1987. He became VP & Medical Director of Phoenix Life in 1989, working with its direct and reinsurance areas, group health and disability. Moving to Merrill Lynch in 1997, he developed an underwriting approach for its older age clientele. In 2001, he joined MassMutual, creating its first electronic underwriting manual and updating its requirements, preferred programs and ratings. He moved home to northern California in 2005 and now mixes ranch work with consulting, including ongoing research work for CRL.

Table 1. Distribution of study population by urine RBC count, urine hemoglobin positivity, age and sex.

Age/sex group	Urine RBCs (cells per HPF)	Urine hemoglobin negative	Urine hemoglobin positive	Total
F 20 to 59	0	1,304,462	33,576	1,338,038
	1 to 4	22,847	24,975	47,822
	5 to 9	5,847	8,661	14,508
	10+	4,047	27,599	31,646
M 20 to 59	0	1,944,100	17,118	1,961,218
	1 to 4	4,226	8,972	13,198
	5 to 9	611	1,503	2,114
	10+	446	2,786	3,232
F 60 to 89	0	110,699	1,390	112,089
	1 to 4	3,278	950	4,228
	5 to 9	798	303	1,101
	10+	444	426	870
M 60 to 89	0	185,003	1,624	186,627
	1 to 4	675	962	1,637
	5 to 9	144	194	338
	10+	119	526	645

Table 2. Cox regression mortality ratios for hemoglobin-positive urine RBCs vs. no RBCs and all levels of hemoglobin (reference)

Age/sex group	Urine RBCs (cells per HPF)	Deaths	Total	Mortality Ratio	95% CI Lower	95% CI Upper
F 20 to 59	0 (reference)	4,685	1,338,038			
	1 to 4	89	24,975	1.116[1]	.904	1.376
	5 to 9	23	8,661	.891	.591	1.342
	10+	70	27,599	.987	.779	1.250
M 20 to 59	0 (reference)	12,564	1,961,218			
	1 to 4	78	8,972	1.203[1]	.962	1.503
	5 to 9	12	1,503	1.056	.599	1.860
	10+	30	2,786	1.554	1.086	2.224
F 60 to 89	0 (reference)	4,586	112,089			
	1 to 4	40	950	1.036[1]	.759	1.415
	5 to 9	10	303	.786	.409	1.512
	10+	28	426	1.663	1.147	2.412
M 60 to 89	0 (reference)	9,277	186,627			
	1 to 4	61	962	1.185[1]	.921	1.524
	5 to 9	15	194	1.350	.813	2.241
	10+	53	526	1.915	1.462	2.509

Table 3. Cox regression mortality ratios for positive hemoglobin levels vs. negative hemoglobin (reference) for applicants with no RBCs

Age/sex group	Urine hemoglobin (μg/dL)	Deaths	Total	Mortality Ratio	95% CI Lower	95% CI Upper
F 20-59	<100 (reference)	4,567	1,304,462	1		
	100 to 499	86	23,583	1.156	.934	1.431
	500 to 999	16	4,635	1.258	.770	2.055
	1000+	16	5,358	1.088	.666	1.778
M 20-59	<100 (reference)	12,430	1,944,100	1		
	100 to 499	113	15,056	1.142	.948	1.375
	500 to 999	8	1,132	1.161	.580	2.322
	1000+	13	930	2.520	1.463	4.341
F 60-89	<100 (reference)	4,514	110,699	1		
	100 to 499	51	1,098	1.114	.845	1.468
	500 to 999	9	145	1.636	.851	3.147
	1000+	12	147	2.407	1.366	4.242
M 60-89	<100 (reference)	9,175	185,003	1		
	100 to 499	69	1,284	1.070	.844	1.356
	500 to 999	14	195	1.384	.819	2.338
	1000+	19	145	2.857	1.821	4.481