

A Protocol for Bladder Cancer Screening and Medical Surveillance among High-Risk Groups: The Drake Health Registry Experience

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In 1986, the Drake Health Registry Study initiated bladder cancer screening for 366 persons at high risk because of occupational exposure to β -naphthylamine. The Drake Health Registry Study screening protocol consists of urinalysis, Papanicolaou cytology, and quantitative fluorescence image analysis. A positive screening test qualifies participants for a full diagnostic evaluation. The screening protocol has been modified during the first 3 years of the program's existence to address unexpected patterns of test results and to incorporate advances in screening technology. The current protocol, which has a two-tiered screening schedule, has been utilized successfully for 15 months. Of the 26 positive results to date most have been based on abnormal Papanicolaou cytology and/or quantitative fluorescence image analysis. Bladder abnormalities were cited among most of the 18 study members who underwent diagnostic evaluation, including chronic cystitis, inflammation, hyperplasia, and dysplasia. We conclude that the screening program is detecting very early changes in a relatively young cohort and that these persons must be monitored over a number of years to ensure adequate medical surveillance.

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0096-1736/90/3209-0881\$02.00/0

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Screening programs for occupationally based bladder cancer are based on a variety of protocols that incorporate tests for hematuria and abnormal cytology. The emphasis assigned each test and the criteria for pursuing more invasive diagnostic procedures vary widely. This paper will describe the development of the clinical protocol currently in use by the Drake Health Registry Study (DHRS). The DHRS screens persons who are at high risk of developing bladder cancer because of possible occupational exposure to the bladder carcinogen, β -naphthylamine.

Background

The Drake Chemical Company and its predecessor, the Kilsdonk Chemical Company, operated in Lock Haven, Clinton County, Pennsylvania from 1940 until 1981. At that time the Drake Company declared bankruptcy and the surrounding 8-acre area, which had also served as a disposal site for the two firms, was designated a hazardous waste site eligible for emergency cleanup under the Comprehensive Environmental Response, Compensation, and Liability Act, popularly known as Superfund.

The Drake plant was one of the few in the United States where the potent bladder carcinogen, β -naphthylamine (BNA), was manufactured.¹ In both Europe and the United States, BNA has been found to be associated with increased incidence of inflammation of the bladder and/or bladder cancer,²⁻⁴ with a relative risk as high as 87 for bladder cancer.² BNA was produced at Kilsdonk for use as an intermediate in the manufactur-

ing of dyes and antioxidants between 1947 and 1962, when its use was banned in Pennsylvania. It is suspected, however, that after 1962 small amounts of BNA may have been synthesized by Drake as a residual product in the manufacture of Broenner's acid, which was produced in large quantities until the plant closed. In addition to BNA, another carcinogenic aromatic amine, benzidine, was also utilized at the plant.

Several studies have provided evidence that the incidence of bladder cancer in the Drake cohort is higher than would be expected, due to the use of BNA and/or benzidine. In 1963 Lieben⁵ found that 11 of 77 cases of bladder cancer reported by Lock Haven area hospitals occurred in relatively young persons working at one of two local chemical companies (including Kilsdonk). In 1983 the Pennsylvania Department of Health (DOH) developed a comprehensive health surveillance program for the Lock Haven area. One component involved an ecologic study of countywide mortality rates between 1950 and 1979⁶ that showed a significantly increased number of bladder cancer deaths among white men in Clinton County. Other components included studies of morbidity and mortality among local residents and among former Drake and Kilsdonk employees and their family members. Although there was no evidence of a serious health problem related to exposure among the community at large,⁷ prevalence rates for total cancers were elevated among BNA-exposed employees.⁸ Finally, as part of the current study, an analysis of the mortality experience of the 408 former employees of Drake or Kilsdonk revealed a greater than 20-fold excess in bladder cancer when comparisons were made with local county rates.⁹

The DOH health surveillance program also included a bladder cancer screening program for persons who worked at the Drake Chemical Company for at least 3 months. In 1986, with the implementation of the DHRS, this screening effort was expanded to include anyone who ever worked at either Drake or Kilsdonk. The DHRS is conducted by the University of Pittsburgh and the Lock Haven Hospital under a cooperative agreement with the DOH and Centers for Disease Control/National Institute for Occupational Safety and Health, with funds from the Agency for Toxic Substances and Disease Registry. Besides the screening program, the DHRS includes a comprehensive epidemiologic study and evaluation of the notification and screening efforts. Details concerning the history of the DHRS and results at the end of its 3rd year are reported elsewhere.⁹

Screening Program and Development of the Protocol

A total of 408 former Drake/Kilsdonk workers has been identified. Of these, 42 died before notification. Of the 366 persons who are considered eligible for the screening program, 261 have been registered. Like the cohort as a whole, the registrants are relatively young (median age 36), and more than 60% were hired after 1970.

Registry participants are provided a battery of screening tests based on a voided urine specimen. The

screening tests consist of a standard urinalysis to check for hematuria (both dipstick and microscopic), a Papanicolaou (PAP) cytology, and a quantitative fluorescence image analysis (QFIA), performed at the University of Oklahoma.¹⁰⁻¹² The QFIA is based on the quantification of DNA contained in the bladder cells collected from the urine. DNA content of each cell is measured from its absolute fluorescent intensity after staining with either acridine orange (AO) or Hoechst dye. The threshold for a positive test is 5C (where 2C is equivalent to a normal diploid cell), which is 2 SDs beyond the fluorescence level produced by normal dividing cells.¹³ Any cell with fluorescence greater than 5C is considered abnormal. The QFIA is the most experimental component of the protocol and can measure very early, precancerous cell changes. Its major strength is its potential for detecting low-grade tumors that are missed by conventional PAP cytology.¹³ Although its efficacy has been established in clinical practice among persons with a history of cancer, its sensitivity and specificity as a screening tool in an asymptomatic population are still being established.

The clinical protocol defining the interpretation and followup for these tests has been modified over the 3 years of the existence of the DHRS in response to observed patterns of outcomes and changes in technology. At the outset of the program, the criteria for a "positive" test (ie, warranting an immediate, more invasive diagnostic evaluation) was based on that used to screen a cohort of BNA-exposed workers in Augusta, Ga¹³ and consisted of one or more of the following: (1) evidence of hematuria in the urinalysis, (2) a PAP cytology result of class II or above (atypical, suspicious, or positive), (3) a QFIA in which at least one cell produced a reading greater than 5C in the AO test.

The diagnostic evaluation consisted of a urological consult, and, if indicated, an intravenous pyelogram followed by cystoscopy with random biopsy. In the initial and all subsequent protocols, the patient is referred for treatment outside the program when a positive diagnosis is made for a tumor.

In the initial protocol, follow-up after the first screening session was determined according to occupational risk group. Persons working before 1966 were included in the high-risk category and were eligible for semi-annual screening. Persons working longer than 5 years after 1966 were considered to be at medium risk and eligible for annual screening. Anyone working for less than 5 years after 1966 belonged to the low-risk category and was eligible for only one screening if the initial results were negative. If the results of the first screen were positive, the person was shifted into the medium-risk group for the remainder of the screening program.

Very early in the program, the protocol was modified in response to an unusual pattern of screening results. Results during the first 3 months of the study showed an unexpectedly high number of positive results among young, low-risk employees. Most of these positive results were based on the QFIA. Concern was raised about pursuing an invasive procedure with these men. At the same time, newly available developments in the QFIA technology had made possible finer, more specific defi-

nitions of a positive QFIA result. The AO test, which measures all double-stranded nucleic acids, was supplemented by the more specific Hoechst test, which measures DNA only. In addition, the laboratory had perfected a semiautomated, computerized method of identifying cells above the 5C threshold, making it possible to increase both the speed and standardization of the analysis. As a result, the definition of a positive result was refined, and a monitor category was added to the protocol. The latter comprised persons with non-negative results that warranted more frequent screening rather than invasive diagnostic evaluation.

The resulting protocol incorporated a visual cytology diagnosis to differentiate between "positive" and "monitor" categories. These criteria are listed in Table 1.

This protocol was used throughout the remainder of the first year of the DHRS.

Current Protocol

At the end of the first year, in October 1987, a critical evaluation was made of the pattern of outcomes seen to ensure that the protocol for periodic rescreening of cohort members was appropriate. Table 2 shows the pattern of clinical outcomes (positive, monitor, and negative) at the end of the 1st year cross-tabulated against occupational risk group. The large proportion of "positive" and "monitor" outcomes among registrants categorized as low risk raised serious concerns about the likelihood of risk-group misclassification. Work records had been sketchy and the study was relying to a large extent on self-reports to classify persons into the appropriate risk group. It was decided to drop the risk-group criterion and make periodic rescreening available to all members of the cohort, regardless of occupational history, for the remaining 4 years of the registry. These changes were instituted in February 1988 and remain as the operating clinical protocol.

The QFIA had undergone further developments that

also were incorporated into the protocol. Beginning in mid-1987, the QFIA was reported in terms of risk groups 1 through 5 (highest to lowest risk). (These risk-group definitions are copyrighted 1987, 1988 by Cytodiagnos-tics, Inc.) The risk groups are based on the ratio of positive cells to each 500 cells examined, a minimum of 500 cells being required for a reliable sample.

The risk groups are determined according to the criteria shown in Table 3.

Risk groups 4 and 5 are considered low risk, risk group 3 is a moderate-risk group, and risk groups 2 and 1 are considered high risk. Under certain circumstances (eg, papillary clusters or a sample of fewer than 500 cells) the criteria are adjusted according to the judgment of the cytologist and laboratory supervisor (R. Bass, personal communication, 1989).

A third change to the protocol was the more precise definition assigned to hematuria. At the same time, the role of physician discretion was formally incorporated into the protocol.

The Figure provides a flowchart of the current DHRS screening protocol. All registrants start on an annual screening cycle. At the screening, a medical history is obtained. If the registrant reports untoward symptomatology, he or she is advised to seek urologic consultation. Hematuria is the second test level. Positive hema-

TABLE 3
QFIA Risk-Group Criteria

Risk Group	Visual Cytology	Cells above 5C Threshold	
		AO	(and/or) Hoechst
1	Suspicious or positive	≥0 per 500	≥0 per 500
2	Atypical	≥2 cells per 500 (≥.4%)	≥2 cells per 500 (≥.4%)
3	Atypical	1-2 cells per 500 (.2-4%)	<2 cells per 500 (<.4%)
4	Atypical	<1 cell per 500 (<.2%)	0
5	Negative	0	0

TABLE 1
Visual Cytology Diagnosis Protocol

Positive	Monitor
1. PAP cytology: atypical, suspicious, or positive and/or	PAP cytology normal and
2. Hematuria (dipstick or microscopic) and/or	1. AO: one cell per 500 above the threshold with visual cytology atypical and/or
3. AO: one or more cells per 500 above the 5C threshold with visual cytology suspicious or positive and/or	2. Hoechst: one cell per 500 above the threshold with visual cytology atypical
4. Hoechst: one cell per 500 above the 5C threshold with visual cytology suspicious or positive or more than one cell per 500 above the 5C threshold.	

TABLE 2
Distribution of Urine Cytology Results by Risk Group at the End of Year 01

Risk Group	Cytology Result			
	Positive, n (%)	Monitor, n (%)	Negative, n (%)	Total, n (%)
High	6 (35.4)	4 (19.0)	12 (18.8)	22 (21.6)
Medium	3 (17.6)	2 (9.5)	10 (15.6)	15 (14.7)
Low	8 (47.0)	15 (71.5)	42 (65.6)	65 (63.7)
Total	17 (100.0)	21 (100.0)	64 (100.0)	102 (100.0)

(or, to allow for persons who do not adhere to scheduled appointments, two non-negative tests in 24 months) he or she is then advised to undergo a diagnostic evaluation. Registrants stay on a semi-annual screening cycle until all tests are negative on four consecutive screening visits.

Diagnostic evaluations (intravenous pyelogram and cystoscopy) are performed on the same person no less than 12 months apart, and *random* biopsies are separated by 24 months (biopsies of suspicious tissue are still performed as indicated).

Screening Status at Month 36 of the DHRS

Table 4 presents the screening status of those currently enrolled according to the number of visits they have completed. At the onset of the 4th year of the registry, 172 persons were assigned to annual screens based on negative test results. For 84 persons, this assignment is based on one screen only, 79 have had two screens, and 9 have returned for a third screening session. Another 47 persons have had at least one test with a monitor level result and are on a 6-month screening cycle. Of the 47, 12 persons had only one screening session, 21 have had two visits, and 14 completed three. (Six persons have been dropped from the screening cycle entirely either because they have died or because of a previous cancer diagnosis).

A total of 26 persons had positive test results on one or more screens since the beginning of the registry. Of these, 18 completed the diagnostic evaluation (one person has undergone two procedures) and now are assigned to semiannual screening. Another seven persons were placed on semiannual screening either because they had refused the cystoscopy or because an invasive procedure was contraindicated by another medical condition. An additional person has since died from an unrelated illness. Table 5 shows the distribution of these positive screening test results according to the protocol criteria. Of the 26 positive results, one was due to untoward symptomatology, one to gross hematuria, and the remaining 24 to PAP cytology or QFIA results. Only three positive results were based solely on an abnormal PAP. Another eight were based on abnormal PAP plus

TABLE 4
Screening Status at Month 36

No. of Screens Completed	Screening Category			Total
	Ever Positive	Ever Monitor*	Always Negative	
1	8†	12	88†	108
2	11	21	79	116
3	7	15†	9	31
Total	26	48	176	250
Dropped†	1	1	4	6
Currently enrolled	25	47	172	244

* Includes those ever "monitor" and never "positive."

† Six persons were dropped from the screening program because of death or prior diagnosis of cancer.

TABLE 5
Distribution of 26 Positive Results According to Protocol Test Results

Symptomatology	1
Hematuria	1
PAP only	3
(PAP ≥ class III)	(1)
PAP and QFIA	8
(PAP ≥ class III)	(3)
QFIA only	13

a positive QFIA result, and 13 on QFIA only. There have only been four instances of PAP scores above class II.

Two persons have had positive results on two consecutive screening visits. One, after his first visit, underwent a cystoscopy with a negative diagnosis. His cytology results have been abnormal for two subsequent screening sessions and he will be scheduled for a second cystoscopy.

With the initiation of third and fourth screens, the protocol determines clinical management based on the pattern of screening results seen over time. Thirty-one persons have completed three screening sessions and four were scheduled for a fourth. Two of these persons had monitor-level or positive results at their first session with two subsequent negative tests. They will revert to annual screening if their next two tests are negative. Conversely, two persons who have had two consecutive monitor-level screening results will be eligible for a diagnostic evaluation with a third.

As noted above, 18 study members have been referred for diagnostic evaluation since the beginning of the DHRS program. In most cases, the diagnosis was of some type of bladder abnormality, including chronic cystitis, inflammation, and hyperplasia. Cytologies performed on bladder wash specimens remained abnormal in eight cases. Two of these procedures were performed after the month-36 cutoff and resulted in one diagnosis of dysplasia and one of carcinoma in situ. Review of these cases by two outside pathologists failed to confirm the diagnosis of carcinoma in situ. The changes seen in the patient diagnosed with dysplasia were considered hyperplasia by the outside reviewers. Nevertheless, both patients are being considered to have had non-negative biopsy outcomes and are being watched closely.

In addition to these suspicious cases based on diagnostic evaluation, the DHRS has identified three deceased and three living study members who were diagnosed with bladder cancer by their personal physicians.

Discussion

At the outset of year 04, the revised clinical protocol had been utilized for 15 months. As noted (Table 5), only 11 positive results were based on PAP cytologies (two additional class II PAPs are yet to be confirmed), including four above class II. Most of the abnormal findings have been based on QFIA scores, which may be detecting very early changes that cannot be treated therapeutically.

In the absence of any confirmed bladder tumors diagnosed at cystoscopy, it is not possible to evaluate the sensitivity and specificity of the screening tests in this cohort. Given the young age of the cohort, however, the absence of detectable cancer is not surprising. It is likely to take several years for symptomatic disease to occur. In fact, for more than 60% of the current registrants, the latent period (hire date at Drake/Kilsdonk until diagnosis or death) has not reached 20 years, the mean latent period of the six bladder cancer cases identified among the cohort. With the initiation of second and third screening sessions, it has been possible to begin identifying persons with consistently abnormal tests who are presumably at high risk for bladder disease, and who should be monitored closely. It should be noted that the two abnormal biopsy results cited above were detected on the basis of their third screening sessions.

The protocol described here was developed to balance the need for early detection of bladder cancer with caution regarding the unnecessary performance of invasive procedures. The two-tiered screening cycle enables the registry to closely monitor persons with abnormal test results without performing a diagnostic evaluation that might be premature in terms of detecting treatable disease. The fact that most abnormalities detected to date are based on the QFIA underscores the long-term nature of the screening process. The use of an early marker like the QFIA in a relatively young cohort enables the early identification of persons at high risk, but also necessitates the commitment to follow these persons throughout the years it may take for them to develop symptomatic disease. As the DHRS approaches the final year of its current funding cycle, an overriding task will be to develop strategies for maintaining screening services beyond the initial 5-year pilot phase.

Acknowledgments

This study was conducted under a cooperative agreement between the Pennsylvania Department of Health, Division of Environmental Health and the Centers for Disease Control/National Institute for Occupational Safety and Health. Funds were provided by the Agency

for Toxic Substances and Disease Registry, Atlanta, Ga. We gratefully acknowledge the advice and assistance of Dr Rebecca Bass of the Department of Pathology, University of Oklahoma. We also acknowledge the efforts of the Lock Haven Hospital project staff: Stephanie Larison, Dr Walter English and Dr S.N. Kapoor, as well as project urologic consultant Dr John Franz.

References

1. Johnson WM, Parnes WD. Beta-naphthylamine and benzidine: identification of groups at high risk of bladder cancer. *Ann NY Acad Sci.* 1979;29:277-284.
2. Case RAM, Hoshier MD, MacDonald DB, Pearson JT. Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediaries in the British Chemical Industry: Part 1. *Br J Ind Med.* 1954;11:75-104.
3. Matanoski GM, Elliot EA. Bladder cancer epidemiology. *Epidemiol Rev.* 1981;3:203-209.
4. Stern FB, Murthy LI, Beaumont JJ. Notification and risk assessment for bladder cancer of a cohort exposed to aromatic amines: III. Mortality among workers exposed to aromatic amines in the last β -naphthylamine manufacturing facility in the United States. *J Occup Med.* 1985;27:495-500.
5. Lieben J. An epidemiological study of occupational bladder cancer. *Acta Union Int Cancer.* 1983;19:759-760.
6. Budnick LD, Sokol DC, Falk H, Logue JN, Fox JM. Cancer and birth defects near the Drake Superfund Site, Pennsylvania. *Arch Environ Health.* 1984;39:409-413.
7. Logue JN, Fox JM. Residential health study of families living near the Drake Chemical Superfund Site in Lock Haven, Pennsylvania. *Arch Environ Health.* 1986;41:222-228.
8. Marsh GM, Constantino JP, Lyons EE, Logue JN, Fox JM. Exposure to the Drake Chemical Superfund Site: morbidity among former employees and family members. *J Environ Health.* 1988;50:389-394.
9. Marsh GM, Leviton LC, Talbott EO, et al. The Drake Chemical Workers Health Registry Study: I. Notification and medical surveillance of a group of workers at high risk of developing bladder cancer. *Am J Ind Med.*, in press.
10. Hemstreet GP, West SS, Weems WL, et al. Quantitative fluorescence measurements of AO-stained normal and malignant bladder cells. *Int J Cancer.* 1983;31:577-585.
11. Hemstreet GP, West SS, and Cook MS. Improved nuclear fluorescence screening technique. *J Occup Med.* 1986;28:10,1004-1010.
12. Bass RA, Hemstreet GP, Honker NA, et al. DNA cytometry and cytology by Quantitative Fluorescence Image Analysis in symptomatic bladder cancer patients. *Int J Cancer.* 1987;40:698-705.
13. Schulte PA, Ringen K, Hemstreet GP, et al. Risk assessment of a cohort exposed to aromatic amines: initial results. *J Occup Med.* 1985;27:115-121.