CONCISE COMMUNICATION

A Large Epidemic of Hepatitis B in Serbia: An Integrated Model for Outbreak Investigations in Healthcare Settings

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We report a comprehensive approach for outbreak investigations, including cluster analysis (Bernoulli model), an algorithm to build inferential models, and molecular techniques to confirm cases. Our approach may be an interesting tool to best exploit the large amount of unsystematically collected information available during outbreak investigations in healthcare settings.

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Hepatitis B virus (HBV) infections have been frequently reported to occur in healthcare settings as a consequence of failure to apply measures for infection control.[1,2] Between August and October 2011, 7 cases of symptomatic HBV genotype D infections occurred among cancer patients from a single outpatient clinic in Vojvodina, Serbia. In November 2011, local authorities decided to initiate an investigation for confirming and describing the outbreak. In December 2011, the activity of the clinic was suspended.

METHODS

The study period (January 15, 2010, through June 1, 2012) was divided into 31 time units (TUs) of 28 days each, including a historical cohort (TUs 0–24) and a surveillance cohort (TUs 25–30).

During November 2011, living patients were tested for hepatitis B surface antigen (HBsAg), HB surface antibody (anti-HBs), and HB core antibody (anti-HBc), and those negative by all tests repeated the tests more than or equal to 6 months after the last admission to the clinic. All patients with HBsAg positivity and/or a sudden alanine aminotransferase (ALT) elevation of grade 2 or higher underwent an HBV DNA assay.[3] Patients' records were reviewed to collect information (Table 1). Levels of ALT/bilirubin (the highest in each TU) were collected retrospectively and during surveillance.

All 31 TUs were used to confirm and temporally locate the outbreak through a Bernoulli one-dimensional model.[4] The model was designed to identify statistically significant grade 2 or higher cluster(s) of either ALT or bilirubin elevations.[3]

The cohort enrolled all patients admitted between TUs 0 and 24 and was used to produce a nested case-control study. Controls were patients negative for HBsAg, anti-HBs, and anti-HBc more than 6 months after the last admission to the clinic. Cases were patients infected with an HBV molecular variant identical to that of at least one other patient. The analysis was conducted by unconditional logistic regression in univariate and multivariate (MLR) models. Exact logistic regression was used when standard logistic regression could not be used.[5] An algorithm was used to select the best set of variables for the MLR model (Table 1). Information about transmission routes were obtained by an auditing procedure and by reviewing healthcare workers' (HCWs') annual HBsAg tests.

HBV serology was conducted by means of commercial kits (bioMérieux). HBV DNA was evaluated using a Real-TM Qual/Ribo-Sorb-64 kit (Sacace Biotechnologies). Molecular analysis was conducted by means of direct sequencing of an 880-bp fragment encompassing aa 48–332 of the reverse transcriptase. Statistical analysis was conducted by means of STATA version 12 and SatScan version 9.1.1.

RESULTS

Between January 15, 2010, and December 15, 2011, 254 patients received chemotherapy at the clinic, of whom 125 (49.2%) were alive and underwent HBV serology tests, 116 (45.7%) had died, and 13 (5.1%) were lost to follow-up. Of the 125 tested patients, 24 (19.2%) were HBsAg positive, 45 (36.0%) were negative by all tests, and 56 (44.8%) were positive for anti-HBs and/or anti-HBc.

Results of cluster analysis (Figure 1) shows that ALT and bilirubin elevations produced significant clusters ($P < .001$) with similar time patterns between TUs 22–27 and TUs 22–26 for ALT and bilirubin, respectively.

Molecular investigation was conducted in 12 of the 24 HBsAg-positive subjects with adequate viral load (sequencing failed in the other 12). The phylogenetic analysis also included 84 HBV sequences from unrelated subjects as background controls for viral variability. All 12 sequences from the outbreak belonged to genotype D, subtype 2, and clustered in a significant monophyletic group (bootstrap 98, 1,000 repetitions).

The case-control study (Table 1) included the 12 cases identified by molecular analyses and 45 controls who tested negative for all HBV serology. The final MLR model provides strong evidence that being admitted in TUs 15, 16, and 21 were the only independent risk factors. In particular, all but one of the cases were actually admitted to the department during these TUs.

The audit procedure identified several breaches in the infection control measures. In particular, the use of personal

TABLE 1. Inferential Model to Assess Potential Risk Factors

	Frequency (step 2)		Univariate analyses (step 3)		Int. MLR models (step 4)		Full MLR model (step 5)		Final MLR model (step 6)	
Variable type (step 1)	Cases	Controls	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Overall	12	45								
Epidemiological baseline										
Male sex	7	10	**5.43 (1.44–20.4)**	**.012**	3.02 (0.54–16.82)	.207	14.77 (0.77–279.80)	.073	8.57 (0.67–110.31)	.100
Age >60 years	6	23	*0.96 (0.26–3.46)*	*.946*	...	...	...	...	...	...
Digestive cancer	7	9	**5.60 (1.17–27.42)**	**.009**	*2.79 (0.52–15.05)*	*.233*	...	...	...	...
Breast cancer	3	22	*0.35 (0.09–1.37)*	*.138*	...	...	...	...	...	...
Diabetes	3	4	3.42 (0.68–17.06)	.131	3.66 (0.59–22.55)	.162	*2.16 (0.07–63.16)*	*.654*	...	...
Piercing	3	19	*0.46 (0.11–1.9)*	*.276*	...	...	...	...	...	...
Drugs										
Fluorouracil	9	20	3.75 (0.93–15.05)	.060	3.90 (0.91–16.76)	.067	9.66 (0.37–250.68)	.1	5.54 (0.44–70.16)	.187
Cisplatin[a]	2	3	*2.80 (0.43–18.19)*	*.277*	...	...	...	...	...	...
Metoclopramide	11	32	4.47 (0.60–33.49)	.142	4.74 (0.53–42.08)	.162	6.20 (0.34–114.83)	.220	7.65 (0.48–122.61)	.150
Cyclophosphan	3	19	*0.46 (0.11–1.9)*	*.276*	...	...	...	...	...	...
Granisetron	6	24	*0.88 (0.24–3.16)*	*.837*	...	...	...	...	...	...
Dexametasone	4	17	*0.82 (0.21–3.19)*	*.777*	...	...	...	...	...	...
Doxorubicin	2	13	*0.49 (0.1–2.54)*	*.393*	...	...	...	...	...	...
Time of admission										
TU 13	2	7	*1.09 (0.19–6.15)*	*.9253*	...	...	...	...	...	...
TU 14	2	6	*1.30 (0.22–7.53)*	*.7677*	...	...	...	...	...	...
TU 15/16[b]	3	4	4.67 (0.89–24.46)	.066	18.29 (0.57–1,559.59)	.124	168.87 (1.72–1,656.53)	.028	**138.73 (2.45–790.54)**	**.017**
TU 17	3	6	*2.17 (0.46–10.23)*	*.3247*	...	...	...	...	...	...
TU 18	2	2	4.30 (0.61–30.48)	.141	3.96 (0.20–753.44)	1.000	*2.11 (0.04–104.47)*	*.707*	...	...
TU 19	4	3	**7.00 (1.5–32.61)**	**.012**	*0.92 (0.20–84.83)*	*1.000*	...	...	...	...
TU 20	8	7	**10.86 (2.9–40.64)**	**.001**	*0.72 (0.04–10.86)*	*1.000*	...	...	...	...
TU 21	8	8	**4.43 (1.20–16.40)**	**.025**	8.95 (0.58–∞)[c]	.114	45.07 (2.27–896.32)	.013	**49.03 (3.17–756.40)**	**.005**
TU 22	8	12	**5.50 (1.49–20.32)**	**.010**	*1.07 (0.00–16.60)*[c]	*1.000*	...	...	...	...
TU 23	8	14	**4.43 (1.20–16.4)**	**.025**	*2.44 (0.12–58.36)*	*.878*	...	...	...	...
TU 24	5	7	**3.88 (1.00–15.10)**	**.049**	3.14 (0.29–51.25)	.512	19.10 (0.61–596.37)	.093	14.22 (0.64–311.93)	.092
Invasive procedure										
Abdominal surgery	6	6	**4.00 (1.08–1.48)**	**.036**	NA	NA	*0.60 (0.01–28.54)*	*.797*	...	...
Mammal surgery	2	17	*0.33 (0.07–1.62)*	*.1681*	...	...	...	...	...	...
No. of admissions	...	...	1.05 (1.00–1.13)	.052	NA	NA	0.90 (0.69–1.18)	...	0.93 (0.76–1.13)	.475

NOTE. The primary set of variables consists of 59 binary variables and 1 continuous variable. Step 1: We divided all the variables into 4 categories, that is, epidemiological baseline characteristics, exposure to intravenous drugs, exposure to invasive procedures, and time of exposure to chemotherapy (25 time units [TUs] of 28 days each). In addition, one variable was considered an a priori confounder and directly included in the final model (ie, number of admissions to the department; continuous). Step 2: As we were seeking potential common exposures between patients, all variables with less than 2 exposed cases were discarded. Step 3: Univariate analyses was performed, and all variables with an odds ratio (OR) less than 3 were dropped. Step 4: Four intermediate (Int.) multivariate logistic regression (MLR) models that included variables within a specific category were established, and all variables with an adjusted OR less than 3 were discarded. Step 5: All variables with an adjusted OR more than 3 in intermediate MLR models were included in the full MLR model. Step 6: The best set of variables to be included in the final MLR model was chosen on the basis of simplicity and fitness criteria through likelihood ratio tests (LRTs) to compare the full model with a simpler model including only variables with an adjusted OR more than 3 in the full model. The simpler model was preferred over the more complex one for LRT $P > .1$. Shown in boldface are risk factors associated with cases with $P < .05$; shown in italics are dropped variables. CI, confidence interval; NA, not applicable.

[a] Cisplatin was always administered with intravenous mannitol and intravenous furesonide (no other patients received intravenous mannitol or furesonide).

[b] The same cases and controls were admitted concurrently in TUs 15 and 16.

[c] Median unbiased estimate.

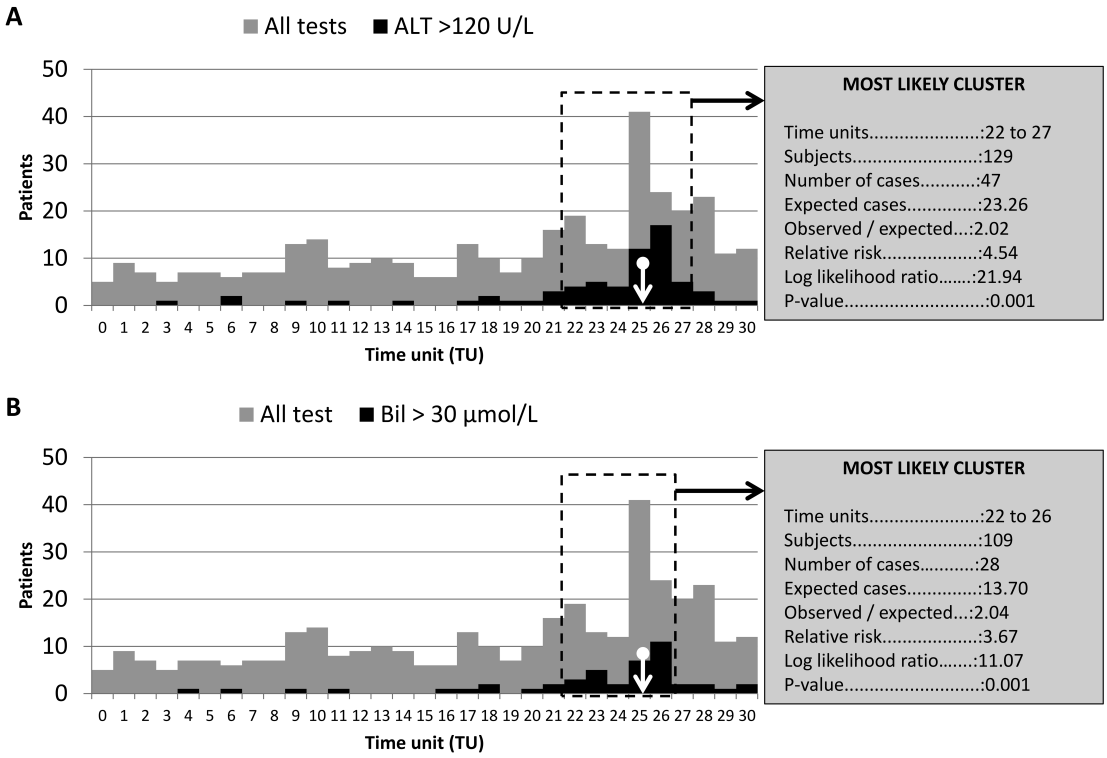

FIGURE 1. Bernoulli model for temporal cluster detection. The white arrows indicate the time when the activity of the clinic was suspended. *A*, Identification of a cluster of alanine aminotransferase (ALT) elevations of grade 2 or higher. Shown in gray are overall tests for ALT determinations (366 tests for 180 patients), and shown in black are determinations of ALT more than 120 IU/L (66 test for 46 patients). Epidemiological and inferential parameters of the cluster are reported in the box. According to the model, the expected risk of ALT grade 2 elevation or higher for the overall period is 18.03 per 100 subjects tested. *B*, Identification of a cluster of bilirubin elevations of grade 2 or higher. Shown in gray are overall tests for ALT determinations (366 tests for 180 patients), and shown in black are determinations of ALT more than 30 μmol/L (47 test for 31 patients). Epidemiological and inferential parameters of the cluster are reported in the box. According to the model, the expected risk of bilirubin grade 2 elevation or higher for the overall period is 12.57 per 100 subjects tested.

protective equipment (glove removal and hand hygiene between patients) was erratic, and patients received therapy in the same room where other patients were sampled. HCWs' serostatus records showed that 1 surgeon among the 31 HCWs who performed abdominal surgery on 3 of 12 of the cases was HBsAg positive. He refused to provide a serum sample for the molecular analyses.

DISCUSSION

This investigation suggests that between September 23, 2011, and March 8, 2012 (168 days between TUs 22 and 27), a large outbreak of HBV infection occurred among the patients who received chemotherapy in the clinic. The transmission was most probably due to a few puncture events that occurred because of poor application of infection control measures in 2 different time periods, either March 11–May 5, 2011 (TU 15/16), or August 26–September 22, 2011 (TU 21). Consistent evidence supports this hypothesis.

First, the Bernoulli model provided strong evidence for simultaneous clusters of hypertransaminasemia and hyperbilirubinemia suggestive of new HBV infections. In fact, as the patient case mix did not change, additional causes of increasing incidence of symptomatic hepatitis (eg, drug toxicity or reactivation of latent infection)[6,7] were unlikely. Second, the molecular analyses indicated that all confirmed cases were infected with an identical HBV genotype D strain that is fairly common in Serbia.[8] Third, the duration of the temporal clusters is well within the expected 180-day distribution of acute HBV infections after a single or a few puncture exposures.[9] Finally, the MLR model indicated that receiving chemotherapy in TU 15/16 and TU 21 were the only independent risk factors. Both of these periods are compatible with the incubation time of HBV and resemble well the delayed enzyme kinetics observed in acute HBV infection in immunocompromised subjects.[10]

Outbreak investigations in healthcare settings represent a peculiar circumstance. Although consequences may be severe because of patients' baseline conditions, an impressive source of information is available for conducting investigations aimed at explaining and preventing similar events in the fu-

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ture. However, this information (mainly contained in patients' records and clinical logs) is often not easy to use and is underexploited. Here we report an investigational approach that integrates classic epidemiology, updated statistics, and advanced molecular techniques to organize information into data and to produce reliable analyses. In particular, the present investigation relies on classic study design, which provided the framework for investigation (cohort and case-control study); an objective system to build the MLR model (a reproducible algorithm for selection of variables); updated inferential models to overcome the limitations of standard logistic regression (exact logistic); and a system to identify and temporally locate clusters of signs suggestive of specific diseases when no preexisting surveillance was implemented (Berunulli model). Finally, phylogenetic analyses were used to confirm the genetic correlation between HBV strains and to produce consistent conclusions.

Despite its limitations (we could not define any specific transmission mode and did not perform formal case-contact tracing), the investigation allowed us to identify and describe the event. We believe that our approach proved to be an interesting tool for retrospective outbreak investigations, and it may make a difference in outbreak investigations where no preexisting surveillance system is implemented and subjects' information is unsystematically collected and mainly represented by those nonspecific signs and symptoms that are usually reported in clinical records.

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