review board and the ethical com-mittes at Dhaka Medical College and Hos-pital, Bangladesh.

Ages of GBS patients ranged from 2 to 65 years (mean, 24 [standard deviation {SD}, 14]), and of ONDC from 4 to 65 years (mean, 24 [SD, 14]). FCs were signifi-cantly older (P < .001) as their ages ranged from 11 to 57 years (mean, 33 [SD, 10]). Seventy-two percent of GBS patients and 74% of ONDCs were male, whereas 47% of the FCs were male. HEV-specific serum immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies were detected with a commercial enzyme-linked immunosorbent assay (ELISA; Wantai, Beijing, China). The IgG sero-prevalence is depicted by age group in Figure 1A. It is in line with earlier reports [5] and illustrates the high prevalence of HEV infection. The mean IgG seroprevalence among GBS patients (44%), ONDCs (46%), and FCs (41%) was similar between patients and controls (data not shown). In contrast, anti-HEV IgM seroprevalence (Figure 1B) was significantly higher among GBS patients as compared to ONDCs (P < .01) and FCs (P < .001). IgM levels directed against other viral pathogens and Mycoplasma were measured as well to control for cross-reactivity (data not shown). IgM seropositive individuals for HEV RNA [6], yielded 1 positive serum sample classified as HEV genotype 1, with a viral load of 6.29 log IU/mL. The sequence identified was deposited into GenBank (accession number KF192078).

These data for the first time show an association between GBS and antecedent HEV infection in a unique case-control study in a developing country. Additional prospective case-control studies should confirm this association, which would add GBS to the disease burden associated with HEV infection. Since poliomyelitis was eradicated from Bangladesh in 2000, GBS has been the most prevalent cause of acute flaccid paralysis. Sporadic cases of imported poliomyelitis are still described and may be clinically misdiagnosed as HEV-associated GBS, emphasizing the need for adequate diagnostic methods to dis-tinguish between these disease entities.

Notes

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Long-term Survival in Community-Acquired Pneumonia Caused by Other Bacteria Than Pneumococci Is Impaired More Than in Pneumococcal Pneumonia: Effect of Underlying Disease?

To the Editor—With great interest we read the study by Sandvall and colleagues [1], in which they report mortality rates up to 32.2%, 10 years after initial recovery from pneumococcal pneumonia. Although this study provides important information and is the largest to date on long-term outcome of microbiologically confirmed pneumococcal pneumonia, we would like to add some comments.

In 2011 we published cause-specific long-term mortality rates of 356 prospectively identified consecutive patients with community-acquired pneumonia (CAP) [2]. In this population, the mortality rate 7 years after initial survival of an episode of CAP was as high as 52.5%, which is in line with other studies reporting mortality rates of 38% to 53% 5 years after CAP [3–5]. Interestingly, the long-term mortality rates shown by Sandvall et al are much lower compared to these previous studies. This can be explained by patient selection bias (ie, only veterans with pneumococcal pneumonia were included in the Sandvall...
original meta-analysis, and 30% (95% CI, −17% to 58%) using a random-effects model. These estimates are similar to those previously reported for the ITTI group, although the 95% confidence intervals are wider.

Notes

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References


Hepatitis E and Guillain-Barré Syndrome

To the Editor—Hepatitis E virus (HEV) infection is the most common cause of acute hepatitis worldwide. Whereas in developed countries it usually presents as a self-limiting disease caused by genotype 3, genotype 1 and 2 infections in resource-limited countries are associated with considerable morbidity and mortality [1]. Besides liver disease, neurologic manifestations may occur, such as Guillain-Barré syndrome (GBS) and brachial neuritis [2]. GBS is the most common cause of acute neuromuscular paralysis in countries where poliomyelitis has been eliminated [3]. GBS patients frequently report preceding gastrointestinal or respiratory illnesses, such as those caused by Campylobacter jejuni, cytomegalovirus, Epstein-Barr virus, and Mycoplasma pneumoniae [4], but in many developing countries antecedent infections have not been investigated. Recent reports on the global burden of HEV infection prompted us to perform a case-control study among GBS patients in Bangladesh, where both HEV genotype 1 infection and GBS are commonly diagnosed [3, 5].

A prospective case-control study was conducted between July 2006 and June 2007 enrolling 100 consecutive GBS cases from Dhaka Medical College Hospital, Bangabandhu Sheikh Mujib Medical University, and Dhaka Central Hospital in Dhaka, Bangladesh. Two controls per case were recruited: one among the family members of the patient living in the same household (family control [FC]); and one was an age-, sex-, and day-matched patient hospitalized in the same ward with another neurologic disease not related to recent infections (other neurological disease control [ONDC]). Written informed consent was obtained from all patients and controls. The project protocol was reviewed and approved by the institutional

Figure 1. Anti–hepatitis E virus (HEV) immunoglobulin G (IgG) and immunoglobulin M (IgM) seroprevalence. A, Percentage of patients and controls within each age group with anti-HEV IgG serum antibodies. B, Percentage of patients and controls with anti-HEV IgM serum antibodies. Abbreviations: FC, family control; GBS, Guillain-Barré syndrome; HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M; ONDC, other neurological disease control.
(H5N2) (M gene), A/chicken/Taiwan/1006/04(H6N1) (HA gene), and A/chicken/Taiwan/TC135/2010(H6N1) (PB1, NP, NA, and NS genes) (Figure 1).

Furthermore, we investigated the molecular signatures of the novel H6N1 virus. One hundred HA and NA gene sequences with higher similarity were chosen for amino acid alignment analysis. For HA, the strain A/Taiwan/2/2013 had a low pathogenicity because its HA1/HA2 connecting peptide region (QIATR/GIF) lacked the multibasic amino acids, which is the signature of highly pathogenic H5 and H7 influenza viruses [4, 5]. In contrast, the sequence before the cleavage site in most H6N1 influenza viruses is QIETR or QIKTR. Most of the mutation sites were in the HA1 part, whereas only 2 changes were found in the HA2 part. Interestingly, in comparison with chicken A/H6N1 viruses, we found 2 unusual mutation sites in the novel human A/H6N1: P200Land A301 T (H6 numbering), which located closely to the domain of HA receptor binding. Moreover, at the critical HA receptor binding site (239–244) (H6 numbering) of this novel H6N1, the position 228 in HA was S, as in human-adapted H3 strains, which effectively raises the possibility that H6N1 might transmit to humans [6]. For NA, the novel virus showed a 12-amino acid deletion in the NA stalk and 5 mutation sites: S31L, G189N, K265R, V389M, and D451E (H6 numbering). The length variation of the NA stalk may affect the host range of influenza A virus [7, 8]. Our results on the origin and molecular characteristics of the novel H6N1 may be useful for future influenza surveillance.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Oseltamivir Effect on Antibiotic-Treated Lower Respiratory Tract Complications in Virologically Positive Randomized Trial Participants

To the Editor—In a meta-analysis of randomized controlled trials, we found that oseltamivir reduced the risk of antibiotic-treated lower respiratory tract complications by 28% (95% confidence interval [CI], 11%–42%) in outpatients and by 37% (95% CI, 18%–52%) in outpatients with laboratory-confirmed influenza infections [1]. This intent-to-treat-infected (ITTI) group included study participants who were confirmed to have influenza infection either serologically (>4-fold or greater rise in hemagglutination-inhibition antibody titer in convalescent sera) or virologically (virus isolation from respiratory samples collected at enrollment). A recent Cochrane review [2] noted that the ITTI group included subjects defined by a posttreatment variable (serology) that might have been influenced by oseltamivir administration, and hence an analysis restricted to the ITTI group might have provided a biased estimate of effectiveness. Because the virus-positive subgroup is the one in which a biological effect would be expected, we have reanalyzed the data from the same trials, restricting consideration to individuals who were culture positive for influenza at the time of enrollment. These data were obtained from Roche for the same trials in our original meta-analysis [1].

Forty-seven percent (1031/2188) of subjects in the oseltamivir arms and 42% (719/1720) of those in the placebo arms had a positive culture at enrollment. Of the 1750 subjects with a positive culture, 4.8% (49/1031) of oseltamivir and 8.3% (60/719) of placebo recipients had an antibiotic-treated lower respiratory tract complication. The pooled reduction in the risk of such a complication for oseltamivir vs placebo was 33% (95% CI, 3%–54%) using a fixed-effect model as in the
Origin and Molecular Characteristics of a Novel 2013 Avian Influenza A(H6N1) Virus Causing Human Infection in Taiwan

To the Editor—On 20 May 2013, the world’s first human-infected case of H6N1 bird flu was reported in Taiwan. A novel avian-origin influenza A(H6N1) virus was confirmed by the National Influenza Center, Centers for Disease Control, Taiwan, and the patient has already recovered. The H6 subtype influenza viruses were first identified in turkeys in 1965, and make up one of the most commonly recognized subtypes in domestic ducks in southern China [1]. Previous studies indicated that the H6N1 virus is low pathogenic [2]. In this study, we investigated the molecular characteristics of the novel avian influenza A(H6N1) virus and performed phylogenetic and coalescent analyses to infer the potential origins.

We first conducted a sequence identical analysis using nucleotide BLAST for all 8 segments of the novel H6N1 virus across 2 Avian influenza virus sequence databases (GISAID, NCBI). The PB1 gene of the novel human influenza A/Taiwan/2/2013(H6N1) (EPI_ISL_143275) has the highest nucleotide sequence similarity to A/chicken/Taiwan/PF3/02(H6N1) at 95.8%, whereas the remaining 7 genes showed that the highest nucleotide sequence similarity to A/chicken/Taiwan/A2837/2013 (H6N1) ranged from 96.2% (NA) to 99.5% (NP). We also constructed the maximum likelihood phylogenetic trees for each genome segment using the program PhyML3.0 [3]. The results of phylogenetic analyses showed that all 8 genes of this virus were clustered in the Taiwan lineage. Taking sampling collection schedules into consideration, the closest relative of A/Taiwan/2/2013(H6N1) is A/chicken/Taiwan/A2837/2013, suggesting the latter might be the precursor of the novel virus.

Our results indicated A/Taiwan/2/2013(H6N1) was reassorted from A/chicken/Taiwan/0101/2012(H5N2) (PB2 and PA genes), A/chicken/Taiwan/A1997/2012

Figure 1. Schematic diagram of origins of the novel reassortant human influenza A(H6N1) virus. The colors of the gene segments in the ovals indicate their origin. Dotted lines represent the different times when the most closely related sequences (identified from phylogenetic analyses) of the novel H6N1 virus were collected.
differences between pairs of samples and would offer more power to the analysis. Further developments are still needed to allow their direct application on clinical samples.

**Note**

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**Antimicrobial Stewardship Education for Medical Students**

TO THE EDITOR—We commend Dr Abbo and colleagues for their study, which highlights the need to standardize and enhance appropriate antimicrobial prescribing and stewardship curricula in US medical student education [1]. Ninety percent of surveyed fourth-year medical students felt that they would like more education on the appropriate use of antimicrobials; only one-third felt adequately prepared to apply principles of appropriate antimicrobial prescribing. The authors found significant heterogeneity in how students from the 3 medical schools accessed appropriate antimicrobial prescribing information. Of concern, the study also identified gaps in medical students’ knowledge regarding antimicrobial management of common infections. Their findings confirm and precisely describe our anecdotal experience that medical students desire, and would benefit from, organized and formal instruction on appropriate antibiotic use.

To help medical schools address this need, Wake Forest School of Medicine, the Centers for Disease Control and Prevention (CDC), and the Association of American Medical Colleges (AAMC) recently developed and piloted an antimicrobial stewardship curriculum for use in US medical schools. This curriculum contains materials for both the preclinical and clinical years of instruction. The preclinical material consists of three 45-minute didactic slide presentations with facilitator notes entitled “Antibiotic Resistance and Its Relationship to Antibiotic Use,” “Get Smart About Antibiotics”: An Introduction to Prudent Antibiotic Use,” and “Common Respiratory Tract Infections: Evaluation and Therapy.” Corresponding exam questions are provided in US Medical Licensing Examination format. Prerecorded audio with slide presentations of each lecture is also available.

For the clinical years, the curriculum contains 5 small-group activities with facilitator guides that are intended for use during family medicine, internal medicine, surgery, pediatrics, and emergency medicine clerkships. The small-group activities highlight antibiotic stewardship principles through case-based scenarios and focus on the appropriate diagnosis and management of common infections where antibiotics are often misused in both the inpatient and outpatient arenas.

The curriculum materials are available for any medical school to use and can be accessed and downloaded free of charge at http://www.wakehealth.edu/AS-Curriculum.

**Notes**

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areas, possibly due to its long history of coevolution with humans [10]. Defense against parasitic infections has been ascribed to many polymorphisms that have been maintained over generations in the human genome. In conclusion, queries about the reasons for increasing unresponsiveness to miltefosine treatment in the Indian subcontinent remain unanswered. Either host and/or parasite multilocus gene polymorphisms might be responsible for the phenomena and should be further ascertained. These findings have important implications for clinical trials of new antileishmanial drugs.

Note

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References


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Reply to Das

To the Editor—In the correspondence [1], Sushmita Das discusses the parasite fingerprinting methods used in our report on increased failure of miltefosine for the treatment of visceral leishmaniasis in Nepal [2]. Fingerprinting is a major support for interpreting studies on treatment efficacy of infectious diseases: the comparison of pathogens present at the onset of treatment and at the time of a new clinical episode aims to distinguish relapse from reinfection, the former being critical for drug efficacy.

This molecular tracking requires a highly discriminatory genotyping method, to increase the power to reject the null hypothesis of identity between the 2 samples. The task is complicated when the pathogen population under study is genetically homogeneous: how to distinguish a relapse from a reinfection with the same genotype? Furthermore, the degree of genetic homogeneity also depends on the genotyping method that is used. Das correctly highlights the possible confusion that can arise from the literature, with some studies reporting genetic homogeneity in Leishmania donovani from the Indian subcontinent (ISC), while others mention the occurrence of genetic polymorphism. A clear definition of genetic polymorphism is needed, and most of all, it should be interpreted in the context of the genotyping method itself. One cannot claim that L. donovani from ISC is polymorphic because of the occurrence of 2 zymodemes in the region [3]; this means only that with multilocus enzyme electrophoresis (MLEE), a method with limited resolutive power, 2 genetic variants were observed in that population. Multilocus microsatellite typing (MLST) is more resolutive and was shown to detect 6 genetic variants in a sample of ISC parasites, whereas in the same sample of strains, multilocus single-nucleotide typing (MLST) evidenced 21 genotypes [4]. There is no contradiction between these different reports, but genetic polymorphisms should be described by comparison with other populations of parasites. For instance, each of the molecular methods mentioned above detect fewer genetic polymorphisms in L. donovani from ISC than in L. donovani from East Africa. Hence, L. donovani from ISC can indeed be considered to be a relatively low polymorphic species: recent findings suggest that this resulted from a recent clonal expansion following an antimalarial DDT campaign in the 1960s [4, 5].

At the time of running our clinical study [2], the most discriminatory method available for direct genotyping in clinical samples (thus without parasite isolation and cultivation, as needed for MLEE, MLMT, or MLST) of L. donovani was kinetoplast DNA polymerase chain reaction–restriction fragment-length polymorphism analysis, previously shown to resolve twice as many genotypes as MLMT [6]. During molecular tracking, nearly identical genotypes were encountered in each patient at the onset of treatment and at the time of relapse, whereas strains with different genotypes were observed between the different patients [2]. The most parsimonious explanation is that the same strain survived the treatment; thus, the patient relapsed. Obviously, an ultimate genotyping method such as whole genome sequencing [5] would probably reveal small
et al (0.2% [1 of 549]) but less than that reported by Rijal et al (9.6% [11 of 115]).

Sixteen children in the cohort were aged <12 and therefore treated with a regimen of 2.5 mg/kg/day. Of these, 5 (31%) relapsed, all in the first 6 months following treatment. This supports the conclusions of a recent paper by Dorlo et al suggesting that the currently applied dose of 2.5 mg/kg/day results in a substantially lower exposure to miltefosine in children than in adults [4].

Our results support Sundar et al’s suggestion that the majority of patients who relapse after miltefosine in India do so within 6 months of treatment. However, in our cohort there were a significant number who relapsed in the 6- to 12-month follow-up period. MSF’s experience with 20 mg/kg Ambisome in Bihar has also shown that >50% of relapses in patients testing negative for HIV occur between 6 and 12 months following treatment (manuscript submitted for publication). As such, we support Rijal et al’s suggestion of longer follow-up for patients treated for VL in the Indian subcontinent, especially considering the move toward a 10-mg single dose of liposomal amphotericin B and lower-dose combination therapies whose longer-term efficacy has yet to be ascertained.

Note

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Multilocus Parasite Gene Polymorphism and/or Parasite-Selected Mutations in Host Genome May Discriminate Between Relapse and Reinfec tion in the Failure of Miltefosine Treatment in Visceral Leishmaniasis

TO THE EDITOR—In their comprehensive cohort study, Rijal and colleagues have explored the occurrence of failure in miltefosine treatment in 120 visceral leishmaniasis (VL) patients in Nepal; they observed an initial cure rate of 95.8% with an alarming relapse rate of 10.8% and 20.0% at 6 and 12 months, respectively [1]. Similar studies in India have reported declines in the success rate of miltefosine therapy in VL patients, the initial cure rate of 97.5% sharply fell to a final cure rate of 90.3% after 6 months, lower than Indian phase 3 trials a decade earlier [2]. Interestingly, the study in Nepal reports no difference between mean promastigote miltefosine susceptibility (50% inhibitory concentration) of isolates between cures and relapses [1].

At this juncture of the elimination program, it is quite pertinent to differentiate between relapse and reinfection in the context of drug efficacy and resistance among Leishmania isolates in the Indian subcontinent. Parasite fingerprinting is required at onset of treatment and at relapse; parasite kinetoplast DNA fingerprinting results were used to conclude relapse over reinfection [1]. Interestingly, using microsatellites as discriminatory markers, genetic homogeneity of Leishmania donovani strains in the Indian subcontinent is reported [3]. In contrast, genetic polymorphisms with 2 zymodemes were reported in L. donovani strains in Bihar, India [4]. Therefore, these contradictory results targeting some specific loci will lead to perplexity.

If other diseases are consulted for reference, drug resistance is common in malaria and tuberculosis. Interestingly, Mycobacterium tuberculosis isolates with identical DNA fingerprinting patterns can possess substantial genomic diversity [5]. Because this heterogeneity is not detained by traditional genotyping, genome sequence–based modeling and experimental evaluation of fluorescent amplified fragment-length polymorphism is a powerful tool for clinical use [6], which might also be helpful to discriminate between disease relapse and exogenous reinfection in VL. Apparently sympatric subpopulations of artemisinin-resistant malarial parasites were reported to contain extremely high levels of genetic differentiation [7]. Therefore, advanced genetic fingerprinting tools are required for Leishmania genotyping, otherwise important information of the disease could be missed or misinterpreted. Recently, whole genome sequencing revealed that miltefosine resistance in Leishmania major mutants can be both genetically and phenotypically highly heterogeneous [8]. Of particular interest, the KALADRUG-R consortium endeavors to develop, authenticate, and propagate new tools for evaluation of drug resistance in Leishmania, one of the focuses being miltefosine resistance (http://www.leishrisk.net/kaladrug).

Conversely, genome-wide association studies of human VL have implicated various host genes and chromosomal loci in disease outcome [9]. Fascinatingly, malaria-selected mutations in human genes promoted parasite survival in endemic...
Correspondence

One-Year Follow-up of Immunocompetent Male Patients Treated With Miltefosine For Primary Visceral Leishmaniasis in Bihar, India

TO THE EDITOR—We read with interest the 2 recent publications [1, 2] investigating the current efficacy of miltefosine in the treatment of visceral leishmaniasis (VL) in the Indian subcontinent. Since 2007, Médecins Sans Frontières (MSF) has been working in Bihar, India, and has treated >9000 patients with VL using liposomal amphotericin B (Ambisome, Gilead Pharmaceuticals). However, in November 2011 MSF experienced a critical shortage of Ambisome lasting 12 weeks. An operational strategy to reserve Ambisome for patients with the most clinically severe disease and women of childbearing age was implemented during this period, which took into account the recommendation of 5 months of compulsory contraceptive coverage for women of reproductive age taking 28 days of oral miltefosine [3], which MSF could not guarantee.

During this shortage, all male patients aged >5 years with a clinical history of primary VL (fever ≥2 weeks with clinical splenomegaly), positive rK39 assay, and negative human immunodeficiency virus (HIV) test who were clinically stable (hemoglobin level >5 g/dL, no obvious coinfections, and able to receive ambulatory treatment) were treated with 28 days of oral miltefosine as per current Indian government guidelines (100 mg daily for >25 kg, 50 mg for ≤25 kg, and 2.5 mg/kg daily for patients <12 years). Treatment was ambulatory with weekly visits for review and drug blister distribution/collection maintained throughout treatment to ensure compliance.

One hundred twenty-four patients were initiated on treatment, of whom 1 (0.8%) died and 4 (3.2%) defaulted during the 28-day treatment regimen. Initial cure was defined as improvement of symptoms, cessation of fever, and >50% reduction in spleen size by the end of treatment. Test of cure was reserved for suspected treatment failures. Initial cure was achieved in all remaining patients (119 [96%]), although 7 patients displayed complete resolution of symptoms by end of treatment without achieving >50% reduction in spleen size.

Final cure was defined as absence of signs and symptoms of VL by 6 and/or 12 months following treatment completion. All patients with suspected relapse had splenic or bone marrow aspirate confirmation of presence of parasites. The final cure results are presented in Table 1. The mean time to relapse was 114 days (SD, 75 days; range, 65-317 days). Of note, 7 of 119 (5.9%) patients relapsed within 6 months of completing treatment, with 2 (1.7%) more relapsing between 6 and 12 months following completion of treatment. Therefore, the total relapse rate at 12 months was 7.6%. None of the patients who failed to achieve >50% reduction of spleen size by end of treatment relapsed. The proportion of patients who had relapsed at 6 months was similar to the 6.8% reported by Sundar et al in Bihar [1], but less than the 10.8% reported by Rijal et al in Nepal [2]. However, in our cohort, the proportion who relapsed between 6 and 12 months following treatment (1.7% [2 of 119]) was higher than that reported by Sundar

Table 1. Six- and 12-Month Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. of Patients</th>
<th>Cure Rate</th>
<th>Cumulative Relapse Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>End of treatment</td>
<td>124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completed and cured</td>
<td>119</td>
<td>96.0% b</td>
<td>. . .</td>
</tr>
<tr>
<td>Death</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Default</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 6 mo</td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cured</td>
<td>111</td>
<td>94% b</td>
<td></td>
</tr>
<tr>
<td>Lost to follow-up</td>
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<td></td>
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</tr>
<tr>
<td>Additional death</td>
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<td></td>
</tr>
<tr>
<td>Relapse</td>
<td>7</td>
<td>5.9%</td>
<td></td>
</tr>
<tr>
<td>After 12 mo</td>
<td>119</td>
<td></td>
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</tr>
<tr>
<td>Cured</td>
<td>108</td>
<td>92.3% b</td>
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</tr>
<tr>
<td>Lost to follow-up</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additional death</td>
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<td></td>
</tr>
<tr>
<td>Relapse</td>
<td>9</td>
<td>7.6%</td>
<td></td>
</tr>
<tr>
<td>Total No. relapsed at 12 mo</td>
<td>9</td>
<td>7.6%</td>
<td></td>
</tr>
</tbody>
</table>

a Patient relapsed before dying.
b Excludes patients lost to follow-up.

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