The efficacy of Bacille Calmette–Guerin (BCG) against pulmonary tuberculosis (TB) in infants and adults varies from 0 to 80% in different studies. However, BCG is generally considered to protect against tuberculous meningitis and miliary TB. When introduced in the 1930s, BCG vaccination was sometimes considered to have a non-TB-related beneficial effect on survival in children. Recent findings have also suggested that BCG may have a non-specific beneficial effect on childhood survival: BCG vaccination was associated with a decrease in infant mortality of 45% in rural Guinea-Bissau and of 40% in Benin. Whether BCG affects specific diseases remains uncertain, but in a case–control study from Brazil, BCG reduced the risk of death from pneumonia by 50%.

The implications of a BCG scar in TB protection and vaccine efficacy has often been discussed, but it is still doubtful whether scar development is linked to the immunological responses.
response to BCG\textsuperscript{14} in spite of studies indicating this.\textsuperscript{17} The immunological relationship between scar and possible non-specific effects of BCG has not been studied. However, considering the importance of vaccination technique for scar development (injected dose\textsuperscript{18} and route of administration\textsuperscript{19}), we assumed that if BCG vaccination has a beneficial non-targeted effect, this effect would be strongest among children who had responded to vaccination with a scar. Consistently, we observed in Guinea-Bissau that among BCG-vaccinated children, a BCG scar was related to better child survival,\textsuperscript{20} and in a hospital study from Malawi a BCG scar was related to less skin infections and sepsis.\textsuperscript{17} The plausibility of BCG being a non-specific immune stimulator has been strengthened by recent studies showing that BCG produces a Th1-biased immune response at birth.\textsuperscript{21} BCG has also been shown to reduce atopy\textsuperscript{22} and cutaneous anergy\textsuperscript{23} and to stimulate both cellular\textsuperscript{23} and antibody responses\textsuperscript{24} to unrelated antigens.

To pursue this pattern, we screened children <5 years of age in Bissau for BCG scars to examine whether better survival for children with a BCG scar was a reproducible observation. We were able to exclude children exposed to TB at home, since we have had a continuous TB surveillance system in the study area. We conducted verbal autopsies (VAs) for children who died in the present and a previously presented cohort study\textsuperscript{20} to identify possible differences in the major causes of death for children with and without a BCG scar.

**Method and subjects**

The study population consisted of residents in four districts of Bissau, the capital of Guinea-Bissau. The districts Bandim I, Bandim II, Belem, and Mindarà have been followed by the demographic surveillance of the Bandim Health Project (BHP) since 1978, 1984, 1984, and 1994, respectively.\textsuperscript{25} Deaths were ascertained by BHP through the routine household visits every third month for 0 to 3-year-old children or through the census system with at least yearly visits for older children.

Two partly overlapping cohorts were used to describe the mortality pattern for children with or without a BCG scar and to describe specific causes of mortality:

**Cohort A.** As part of a two-dose measles vaccine trial, children were recruited at 6 months of age and at the same time screened for the presence of a BCG scar. Between November 1996 and May 1998 we included 1813 children (1676 with scar, 137 without scar) who were BCG-vaccinated at least 1 month before scar reading. As described elsewhere,\textsuperscript{20} scar-positive children had a lower mortality than scar-negative children the following 12 months, the mortality ratio (MR) being 0.41 (95% CI 0.25–0.67).

**Cohort B.** Between January 1998 and October 1999, 1679 children between 3 months and 5 years of age were visited in their homes and examined for the presence of a BCG scar (1194 scar, 485 no scar). This was done as part of a scar survey, in which all houses in Bandim I and Bandim II were visited. The surveillance system was used to identify individuals in the households, and all individuals living in the study area who consented to take part in the study were assessed for vaccine scars. In the year following the scar reading, 1673 of the 1679 children were visited at least once to assess mortality while 6 (4 scar, 2 no scar) children were lost to follow-up. Of these 1673 children, 1617 (1159 scar, 458 no scar) had a documented BCG vaccination at least 1 month before scar reading. The other 56 children were either not vaccinated or the BCG status was unknown.

**BCG vaccination**

Information on vaccination status was obtained from the vaccination card, either at the time of scar assessment (cohort A) or through the routine surveillance system of the BHP (cohort B). In Bissau, the BCG vaccine is customarily given at birth or as soon as possible after the first health contact. BCG immunization was administered Monday to Friday at the central hospital and on Monday and Friday at the local health centre. Pasteur Mérieux, France, and Statens Serum Institut, Denmark, provided the BCG vaccine via the local Expanded Programme on Immunization (EPI). Following EPI recommendations, BCG was administered by a 0.05 ml (0.1 ml for children >1 year of age) intradermal injection in the left deltoid area. For the present study, children were considered BCG-vaccinated if vaccinated at least 30 days before scar reading.

**TB surveillance**

Since May 1996, we have registered all cases of TB in the study area through a TB surveillance system. This was based on passive and active case finding, where all adult patients (≥15 years) living in the study area reporting to a health centre with symptoms or signs of active TB were further investigated. In addition two nurses visited houses, where cases had been found, every third month to detect secondary cases.\textsuperscript{26} Since all scar readings in the present study were done from 1996 onwards, we were able to control for exposure to TB in the household. As recommended by the International Union Against Tuberculosis and Lung Disease,\textsuperscript{27} patients with clinical signs, symptoms, and X-ray changes compatible with active intrathoracic tuberculosis, but without positive bacteriological tests, were treated with antibiotics (co-trimoxazole or amoxicillin) and then re-evaluated clinically and also with a chest X-ray. If there was no improvement and suspicion remained, the patient was diagnosed as having presumed TB. In the present analysis, TB cases include both smear-positive and smear-negative patients with presumed TB. In order to assess the quality of the TB surveillance in the study area, VAs were performed for all deceased adults in the study area from 1996 to 1998. Only two persons who might have died of TB, but had not been diagnosed and treated, were found.\textsuperscript{20}

**Mortality**

Overall mortality for children with a documented BCG vaccination before scar assessment was described for children in cohort B in the same manner as it has earlier been described in cohort A.\textsuperscript{20} Of the 1617 children in cohort B with documented BCG vaccination at least 1 month before scar reading, 488 had also been included in cohort A. If they were enrolled in cohort B before cohort A (31 scar, 8 no scar), their follow-up was terminated in cohort B on the day of inclusion in cohort A. The 449 (416 scar, 33 no scar) children included in cohort A before being examined as part of the cohort B household survey were excluded from cohort B in order not to replicate results. If the scar status did not correlate between the two assessments made in the two different cohorts, the scar assessment relevant for the follow-up time was used. Children were included in
the survival analysis on the day of scar reading or assessment of the vaccination card, whichever came last, and were excluded on the day of moving from the study area or 365 days after scar reading, whichever came first. One child (no scar) dying in an accident was excluded from the analysis. Thus, 1-year mortality from the time of scar reading and onward was described for 1167 (794 scar, 373 no scar) children in cohort B in a Cox regression analysis with age as underlying time (Figure 1). A combined estimate of 1-year mortality for both cohort A and B was based on 2693 person years of observation, not replicating any follow-up time is also presented (Figure 1).

The survival analyses controlled for background factors with possible influence on child mortality including sex, year of birth (1993, 1994, 1995, 1996–97), season of vaccination (rainy: July–November or dry:December–June), district of residence (Bandim 1 or Bandim 2), place of birth (home, the central hospital, or the Bandim health centre), electricity in house (yes or no), schooling of mother (0, 1–4 or >5 years), ethnic group (Pepel, or other ethnic group), and age at vaccination (0–30 days, 31 days or older). In univariable and multivariable Cox regression analyses, these background factors were tested for association with mortality in the joint cohort of A and B, and factors with a \( P \)-value of <0.20 in the multivariable analysis were adjusted for in all multivariable analyses. TB infection had no impact on the results in cohort A,20 and to assess the possible impact of TB infection on our estimate in cohort B, we excluded

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**Figure 1** Schematic description of study years, age and size of the populations in two partly overlapping cohorts (A and B) included in the study of BCG scar and mortality, Guinea-Bissau 1996–2002

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- **Period of inclusion (scar reading) and follow-up**
- **Period of only follow-up**
- 39 children were included in cohort B before inclusion in cohort A: the follow-up time in cohort B was censored at inclusion in cohort A in order not to replicate results
- 449 children were included in cohort A before cohort B and were therefore excluded from cohort B
- 1 child has been excluded from survival analysis due to death in accident
- **Algorithm for cohort A:** \((1325 + 449 + 39) = 1813\) children included, which has been described with regard to BCG scar and mortality elsewhere (20)
- **Algorithm for cohort B:** \((1616 + 449) = 1167\) children included, for whom the relation between BCG scar and mortality is described in the present study
- **Algorithm for cohort A + B:** \((1813 + 1167 – 39) = 2941\) children with a maximal follow-up of 365 days and a mean of 334 days to give in total 2693 years of observation time.
specific mortality as assessed by VA
Of the children with BCG vaccination at least one month before scar assessment, 488 children were included in both cohort A (1813) and B (1617) resulting in a total of 2942 children (2434 scar, 508 no scar) eligible for assessment of different causes of mortality for scar vs no scar. Within the year following scar reading 149 children died (116 scar, 33 no scar). A VA was developed on the basis of a standard VA recommended by WHO58 and adjusted to the local culture and language through discussions and trials together with physicians and a midwife used to perform VA in the study area.29,30 Once the war was over in Guinea-Bissau, a Guinean physician conducted the VA by visiting the relatives of the deceased children between June 2000 and June 2002, the median time from death to interview being 2.4 years. In order to be considered a reliable informant to the VA, the relative had to have been a member in the same household as the deceased child during the time of the child's disease and death. Of the 149 deaths, we were able to conduct a VA for 77% (90 scar, 25 no scar); for the remaining 34 deaths (26 scar, 8 no scar), all close relatives had moved or died when screening started. The physician conducting the VA was blind to whether the child had had a BCG scar or not. When the 115 VAs were carried out, a group consisting of the interviewing doctor, a paediatrician and another medical doctor, being blind to scar status of the children, decided on one of the following diagnoses as probable primary cause of death; accident/war, anaemia, malnutrition, acute diarrhoea, chronic diarrhoea, pneumonia, malaria, measles, meningitis/encephalitis, other disease, fever unclassified and cause unclassified. To simplify presentation, we categorized the diagnoses that contributed <5% to the total causes of deaths as 'other disease/cause', except for one death caused by accident, which was excluded. The expert group deciding on the most probable cause of death took into consideration the whole patient history that could be derived from the VA interview, including duration of a number of symptoms, duration and kind of treatment provided or stopped, and diagnostic tests reported (such as a blood smear test for malaria). The causes of death contributing to ≥5% of the total number of deaths were: malaria (fever, convulsions, affected responses of the child), pneumonia (cough, difficult breathing, fast breathing, chest in-drawings), acute diarrhoea (liquid and watery stools <14 days, dehydration), chronic diarrhoea (liquid and watery stools ≥15 days), and meningitis/encephalitis (fever, stiff neck/bulging fontanelle). To demonstrate whether there was a different distribution of scar status among the dead children in whom we did not succeed in performing a VA, these deaths were presented as 'no VA'. We calculated the risk of dying from each specific disease, according to scar status in the year following scar assessment. This was done through a separate dataset for each diagnosis, where the event was death and where follow-up was terminated at death due to all other causes, thereby creating a cause-specific hazard function.31 Hence, the risk of dying from a particular disease was described in different Cox regression analyses with age as underlying time, one for each major category of disease.

Statistical software
The analyses were done using Stata 7 for Microsoft Windows.
children in high mortality areas. The general distribution of
suggest that it is important to monitor BCG scarring among
is comparable with earlier findings. The observation would
with a BCG scar compared with children without (Table 2). This
following scar assessment was more than halved for children
In this study we have shown that the mortality in the year
lower for scar-positive children [MR 0.42 (95% CI 0.21–0.82)].

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Scar</th>
<th>No scar</th>
<th>Univariablea</th>
<th>Adjustedb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deaths</td>
<td>% Py</td>
<td>Deaths</td>
<td>% Py</td>
</tr>
<tr>
<td>Total</td>
<td>14/794</td>
<td>1.8</td>
<td>719</td>
<td>13/373</td>
</tr>
<tr>
<td>Boy</td>
<td>3/405</td>
<td>0.7</td>
<td>363</td>
<td>6/189</td>
</tr>
<tr>
<td>Girl</td>
<td>11/389</td>
<td>2.8</td>
<td>356</td>
<td>7/184</td>
</tr>
</tbody>
</table>

Excluding TB in household

Total          9/678 1.3 608 10/324 3.1 289 0.39 0.16–0.97 0.37 0.15–0.92
Boy            3/350 0.9 310 4/166 2.4 150 0.38 0.09–1.71 0.36 0.08–1.63
Girl           6/328 1.8 295 6/158 3.8 139 0.37 0.12–1.18 0.37 0.12–1.18

a Cox regression analysis with age as underlying time.

b Multivariable Cox regression analysis adjusted for sex and ethnic group; the significant (P < 0.20) background factor associated with mortality in the largest
dataset through combining cohort A (presented earlier) and B (presented above).

c Mortality ratio.
d Person years of follow-up time.

The cause-specific mortality rate of malaria was significantly
lower for children with a BCG scar than without: MR being 0.32
(95% CI 0.13–0.76). Even if deaths due to malaria were
combined with deaths due to unclassified fever (as these might
well be due to malaria), the mortality rate was still significantly
lower for scar-positive children [MR 0.42 (95% CI 0.21–0.82)].

Discussion

In this study we have shown that the mortality in the year
following scar assessment was more than halved for children
with a BCG scar compared with children without (Table 2). This
is comparable with earlier findings. The observation would
suggest that it is important to monitor BCG scarring among
children in high mortality areas. The general distribution of
causes of deaths corresponded to what have been described
previously from Guinea-Bissau. There were no major
differences in the impact of BCG scarring for different causes of
deaths. The present study had limited power to detect
differences between causes of death due to the small number of
children without a scar, but nothing suggested that such
differences might be important. The numbers were too small for
a comparison of the potential impact of BCG scarring on the
distribution of causes of deaths in the two cohorts, but then
again nothing indicated differences of importance between the
cohorts (data available on request). The MR in the combined
cohort for children with a BCG scar was significantly lower only
for malaria, which was the largest group of deaths together with
‘fever unclassified’. While reasoning about different effects of
BCG on particular diseases, the limitation of the VA method in
determining an exact cause of death should also be stressed.
Even though the recall period was delayed due to the outbreak
of civil war, the physician carrying out the VA did not find the
relatives of the deceased child to have difficulties in
remembering the events leading to the death of the child. This
observation cannot be validated and despite maternal recall of
death of children having been considered quite reliable even
years after the child’s death, the delay is a potential limitation
determining an exact cause of death. The method of VA
may often be the best method available and it is used to
provide approximate values for relatively common causes of
death. Hence, the question of what diseases are most affected
by having a BCG scar remains unsolved. However, in settings such as that of the present study,
VA may often be the best method available and it is used to
provide approximate values for relatively common causes of
death. We therefore believe that our study suggests that the
effect of a BCG scar may be related to a general enhancement
of the immune response to many infections rather than
providing protection against specific infections. This possibility
is supported by studies of the impact of vaccination status on
the case-fatality rate at the paediatric ward in Bissau; non-
specific effects of measles and DTP vaccines were documented,
but there were no differences in the causes of deaths for
vaccinated and unvaccinated children.
There were differences in the prevalence of BCG scar between the two cohorts. These could presumably be explained by the different study procedures since scar assessment could vary between readers; in one study a medical doctor knowing the BCG vaccination status assessed the scar as part of a medical examination in a health centre while in the other study, trained research assistants performed the scar reading in the homes of the children often without access to the vaccination card of the child. BCG scar prevalence varies with age of vaccination and time of scar reading after vaccination.\(^{12}\) For BCG vaccinations given within one month of birth, the prevalence of a recognizable BCG scar will decline over time.\(^{13}\) This could only partly explain the lower prevalence of scarring in cohort B (Table 1). Of the 44 children with a BCG scar despite no documented BCG vaccination, 20% were probably due to a rapid scar reaction since vaccination had taken place within 30 days before BCG scar assessment; the remaining were due to failure to mark BCG vaccination on the vaccination card, misclassification of BCG scar or misclassification of BCG vaccination while inspecting the vaccination card. Since BCG vaccination took place in different years for the different cohorts, the differences in scar prevalence may also be related to changes in vaccination technique, vaccine type, vaccinating nurse, or lack of BCG vaccine during certain periods, resulting in delayed vaccination; we have observed that all of these factors may have an impact on BCG scarring (authors’ unpublished data).

When assessing the relationship between mortality and BCG scar responses, HIV-1 could be a major confounder considering that HIV-1 infection has been shown to suppress scar reaction to BCG.\(^{35,36}\) During the study period (1993–98), HIV-1 infection was increasing in the study area, from 0% in the late 80s\(^{37}\) among individuals \(\geq 15\) years of age to 2.2% in 1996.\(^{38}\) Expecting a vertical transmission rate of 25%,\(^{39,40}\) we estimate that <1% of the children may have been HIV-1-infected in the present study. Considering the numerous beneficial effects on survival with a BCG scar observed in our study and the relatively low prevalence of HIV-1 infection, we reason that even if HIV-1 infection may have had a modifying effect on our results, it could not explain the major survival benefit observed to be associated with a BCG scar. It was shown consistently in cohort A, that a positive tuberculin skin test (TST) was strongly associated with better survival in the 12 months following TST testing and that excluding deaths of children seropositive to HIV-1 did not change the estimates.\(^{20}\)

One could argue that the results of the present study may be skewed as early infant deaths (<3 months) were not included. However, this ought not to be so, unless BCG has a negative effect on survival for the weak and young children which recent findings indicate is not the case.\(^{41}\) Also, since all children included in the survival analyses were BCG vaccinated, confounding due to healthier children being more likely to be vaccinated is not applicable. Instead, it could be speculated that the beneficial effect of having a BCG scar was merely a marker of some children having stronger immune responses and therefore better survival. However, our studies in Guinea-Bissau have clearly found that BCG scarring depends on type of vaccine and vaccination technique. Furthermore, BCG vaccination has been found to be associated with non-specific enhancement of both antibody and cellular immune responses.\(^{23,24}\) Hence, the beneficial effect may in fact be related to the immune response induced by a correctly given BCG vaccination. Future studies should examine the ability of scar-negative children to develop a scar through re-vaccination. This may well be possible since the lack of scarring could be a result of faulty vaccination technique. If the immune stimulation producing a scar, upon re-vaccination, is comparable with the stimulation producing a scar after a primary vaccination, re-vaccination of scar-negative children might reduce infant mortality substantially, depending on the prevalence of scar-negative children. Of interest is also the reason for scar-failure: clearly a child never developing a scar after BCG vaccination may differ from a child with a waned scar reaction. This is also a possible uncontrolled confounder in the present study, although probably

### Table 3 The risk of dying from a particular disease 12 months after scar reading according to scar status among BCG-vaccinated children as assessed by VA, Guinea-Bissau 1993–98

<table>
<thead>
<tr>
<th>Cause of death(^{a})</th>
<th>Scar</th>
<th>No scar</th>
<th>MR (95% CI)(^{d})</th>
<th>MR (95% CI)(^{e})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n/\text{ppy})</td>
<td>(n/100\text{pyc})</td>
<td>(n/\text{ppy})</td>
<td>(n/100\text{pyc})</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>7/2232</td>
<td>0.31</td>
<td>2/457</td>
<td>0.44</td>
</tr>
<tr>
<td>Malaria</td>
<td>20/2232</td>
<td>0.90</td>
<td>8/457</td>
<td>1.75</td>
</tr>
<tr>
<td>Meningitis enceph.</td>
<td>6/2232</td>
<td>0.29</td>
<td>3/457</td>
<td>0.66</td>
</tr>
<tr>
<td>Acute diarrhoea</td>
<td>11/2232</td>
<td>0.49</td>
<td>2/457</td>
<td>0.44</td>
</tr>
<tr>
<td>Chronic diarrhoea</td>
<td>10/2232</td>
<td>0.45</td>
<td>2/457</td>
<td>0.44</td>
</tr>
<tr>
<td>Fever unclassified</td>
<td>24/2232</td>
<td>1.08</td>
<td>4/457</td>
<td>0.88</td>
</tr>
<tr>
<td>Other disease/cause</td>
<td>12/2232</td>
<td>0.54</td>
<td>3/457</td>
<td>0.66</td>
</tr>
<tr>
<td>No VA(^{f})</td>
<td>26/2232</td>
<td>1.17</td>
<td>8/457</td>
<td>1.75</td>
</tr>
<tr>
<td>Total</td>
<td>116/2232</td>
<td>5.20</td>
<td>32/457</td>
<td>7.00</td>
</tr>
</tbody>
</table>

\(^{a}\) Causes of death contributing to 5% or more of the total number of deaths, all other causes are categorized as ‘Other disease/cause’.

\(^{b}\) Number of deaths per person years of follow-up.

\(^{c}\) Number of deaths per 100 person years.

\(^{d}\) MR, as assessed by different Cox regression analyses for each cause of death, where age was underlying time, the event was death within one year after scar assessment from that particular disease.

\(^{e}\) MR adjusted for sex and ethnic group: risk factors associated \((P < 0.20)\) with mortality in the total survival analysis of cohorts A and B.

\(^{f}\) No VAs were carried through among these children, because all household members who had been present during the disease and death of the child had moved or died.
not of major importance considering the relatively low rate of waning of the scar reaction (Table 1).

Larger studies relating BCG vaccination, BCG scar, and other markers of BCG vaccination to specific morbidity, immune profiles, and mortality are warranted in order to determine the immunological mechanisms involved and to assess possible modifications in the current vaccination programme. If BCG has non-specific beneficial effects it should be recommended to re-vaccinate children without a BCG scar, especially considering that the beneficial effect of a BCG scar on survival seems to persist through early childhood.

Acknowledgements
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A.R., P.A. and M.S. planned the study; P.G. conducted the scar survey in cohort B and supervised the tuberculosis-surveillance programme; A.N., M.S. and A.R. constructed and conducted the VAs; A.P., A.N. and A.R. assessed the VA conducted; M.L.G. carried out the scar survey and survival analyses in cohort A; Q.D., P.A., Am.R., and A.R. did necessary extra follow-up and data cleaning; A.R., P.A., and H.J. performed the analyses; A.R. did the first draft of the paper and the all authors contributed to the final version of the paper. A.R. is guarantor for the paper.

KEY MESSAGES
• BCG may have a non-specific beneficial effect on child survival.
• A BCG scar is a marker of better survival among children in countries with high child mortality, an effect that persists through childhood.
• BCG vaccination may affect the response to several major infections including malaria.
• A possible non-specific beneficial effect of BCG could be of large importance and should be studied further in relation to current vaccination programmes.

References


