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Analysis of factors associated with development of Bacille Calmette–Guérin inoculation site change in patients with Kawasaki disease

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Abstract

Objective: The present study was performed to identify factors associated with a Bacille Calmette–Guérin (BCG) inoculation site change in patients with Kawasaki disease (KD).

Methods: Among patients who had received BCG vaccination and treatment for KD at our hospital from 2005 through 2016, 177 patients born in 2005 through 2016 were enrolled. The patients were divided into those with (n = 83, change group) and without (n = 94, no-change group) a BCG site change, and the patient demographics, clinical severity, blood examination results, and echocardiographic findings were compared between the two groups.

Results: The change group was younger at onset and had a shorter interval from vaccination to onset. A BCG site change was observed in patients who developed the onset of KD symptoms from 31 to 806 days after BCG vaccination. Multivariate analysis showed that the interval from vaccination was closely and positively associated with the BCG site change (hazard ratio = 0.995, 95% confidence interval = 0.993–0.997).

Conclusion: A BCG site change in patients with KD is most closely associated with the interval from BCG vaccination to onset.

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Introduction

In Japan, Bacille Calmette–Guérin (BCG) vaccination is currently recommended for infants aged 5 to 8 months. Previously, when producing the BCG vaccine,[1] the pellicles were removed from cultured *Mycobacterium bovis* BCG Tokyo 172 strain to generate moist bacteria, which were then triturated and loaded onto $\leq$15% (w/v) sodium glutamate solution followed by freeze-drying. The freeze-dried samples were mixed with physiological saline to make 80-mg/mL suspensions. Each suspension was applied to the midpoint of the lateral surface of the upper arm, and the vaccine was administered intradermally using a disposable device with 9 needles according to the multipuncture method (18 needle scars). The BCG vaccination program in Japan has changed over time; the current program, in which the vaccine is given during infancy, has been implemented since 2005.

Kawasaki disease (KD) is a febrile illness of unknown etiology affecting more than 10,000 infants per year in Japan. KD is diagnosed based on six principal symptoms and supplemental criteria as described in the Diagnostic Guidelines for Kawasaki Disease.[2] A BCG inoculation site change is one of the supplemental criteria and is considered an effective diagnostic criterion because of its high specificity for KD in Japan, although its cause and associated factors remain unidentified.[3]

Although some studies have shown that age at onset[2] and disease severity[4,5] are major causal factors, none has shown strong correlations between these factors and KD. The aim of this study was to identify factors associated with the development of a BCG site change in patients with KD by analyzing clinical findings in patients who developed KD after BCG vaccination.

Patients and methods

Among patients who had received BCG vaccination and underwent acute-phase treatment for KD (including possible/probable cases) after the first onset of symptoms at our hospital from 2005 through 2016, those who were born in 2005 through 2016 and started treatment before hospital day 7 were enrolled in this study. The patients were divided into those with (change group) and those without (no-change group) a BCG site change. The following factors were compared between the two groups: (1) demographics, including the male/female ratio, age at BCG vaccination, age at onset, and interval from BCG vaccination to onset; (2) severity of symptoms, including the presence/absence and number of principal symptoms and the number of patients with a confirmed diagnosis of KD; and (3) severity of abnormal examination results, including blood examinations [white blood cell, neutrophil, and platelet counts; hematocrit; and concentrations of albumin, aspartate aminotransferase, alanine aminotransferase, sodium, total bilirubin, C-reactive protein (CRP), ferritin, and brain natriuretic peptide] and left ventricular ejection fraction on echocardiography. The groups were

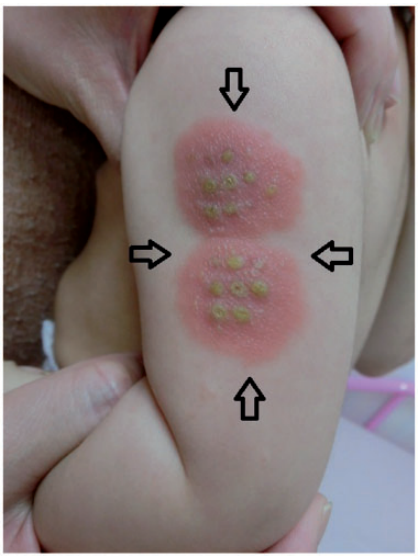

Figure 1. Change in BCG inoculation site in a patient with KD: localized erythema in the shape of the BCG scar (open arrow). BCG, Bacille Calmette–Guérin; KD, Kawasaki disease.

also compared for resistance to high-dose gamma-globulin therapy (2 g/kg per dose) and the presence or absence of acute-phase coronary lesions and coronary artery sequelae.

A BCG site change was defined as localized erythema appearing as a BCG scar (Figure 1). The severity of abnormal examination results was determined based on the worst values obtained before the day of diagnosis. In addition, to determine the contribution of host-related factors, relevant factors for nine patients with recurrent KD symptoms were compared between the first and second onset of symptoms. Data on clinical characteristics and severity of abnormal examination results were compared using Student's t-test. The principal symptoms were compared between these two groups by the χ^2 test. The significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS 15.0J (SPSS Japan Inc., Tokyo, Japan).

This study was approved by the Institutional Ethics Committee (28–40) of Fukuyama Medical Center, Hiroshima, Japan and conducted in accordance with the Declaration of Helsinki. The parents of each infant provided written informed consent prior to participation in the study.

Results

Patient demographics

The patient demographics are shown in Table 1. In total, 177 patients were enrolled in this study. The change group comprised 83 patients, and the no-change group comprised 94 patients. No significant differences in the male/female ratio or age at BCG vaccination were observed between the two groups. Age at onset was significantly lower in the change group, with the oldest patient aged 3 years 2 months ($p < 0.001$). The interval from vaccination to symptom onset was significantly shorter in the change group ($p < 0.001$). There was a strong correlation between age at onset and the interval from vaccination to onset ($r = 0.999$, $p < 0.01$).

The distribution of the interval from vaccination to onset in both groups is shown graphically in Figure 2 and tabulated in Table 2. The shortest and longest intervals in the change group were 31 and 806 days, respectively. A total of 100 patients between both groups experienced symptom onset during the same period, of whom patients in the no-change group accounted for 21% (21 patients). Among the patients with disease onset in the early period after vaccination, all 4 patients with symptom onset within 30 days were in the no-change group while all 8 patients with onset between 31 and 60 days were in the change group. Moreover, all 69 patients with onset $\geq$807 days after vaccination were in the no-change group.

Table 1. Patient demographics and clinical severity.

	Change group	No-change group	p-value
No. of patients	83	94	
Male/female ratio	1.13	0.96	0.59[a]
Age at BCG vaccination (days)	132 ± 43	122 ± 38	0.11[b]
Age at onset (years)	1.2 ± 0.6 (0.3–3.2)	3.5 ± 1.9 (0.3–8.8)	<0.001[b]
Days after vaccination	300 ± 216 (31–806)	1185 ± 679 (1–3108)	<0.001[b]
Fever	83 (100)	94 (100)	
Bilateral nonexudative conjunctival injection	70 (84)	79 (84)	0.96[a]
Change in lips and oral cavity	76 (92)	89 (95)	0.41[a]
Polymorphous exanthema	77 (93)	79 (84)	0.07[a]
Changes in the extremities	74 (89)	86 (91)	0.60[a]
Cervical lymphadenopathy	27 (33)	67 (71)	<0.001[a]
No. of principal symptoms	4.9 ± 0.8	5.2 ± 0.8	0.001[b]
No. of patients with a confirmed diagnosis	65 (78)	81 (85)	0.17[a]

Values are presented as n, n(%), mean ± standard deviation, or (minimum–maximum).
[a]χ^2 test, [b]Student's t-test.

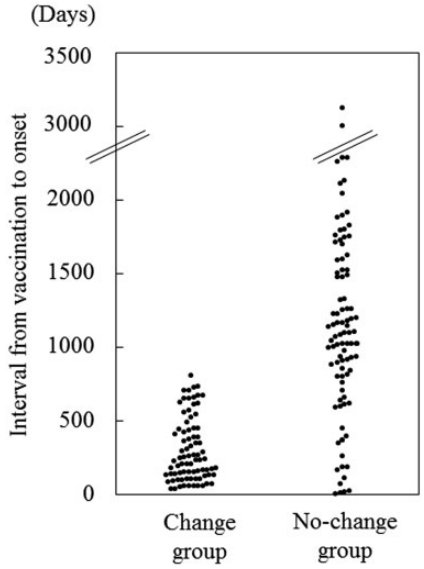

Figure 2. Distribution of interval from vaccination to onset in the two groups.

Severity of symptoms

Table 1 shows the severity of symptoms in the two groups. Among the principal symptoms of KD, cervical lymphadenopathy was observed in 33% and 71% of the patients in the change and no-change groups, respectively, with a significantly lower incidence in the change group ($p < 0.001$). No significant differences were observed in the incidence of any of the other five principal symptoms. The mean number of principal symptoms was significantly higher in the no-change group (5.2 ± 0.8) than in the change group (4.9 ± 0.8) ($p = 0.001$), but no significant difference was observed in the number of patients with a confirmed diagnosis (78% in the change group and 85% in the no-change group).

Table 2. Interval from vaccination to disease onset.

Days after vaccination	Change group	No-change group
0–30	0	4
31–60	8	0
61–806	71	21
≥807	0	69

Severity of abnormal examination results

Table 3 shows that the change group had significantly lower neutrophil counts ($p < 0.001$) and CRP levels ($p < 0.001$) and

Table 3. Severity of abnormal examination results.

	Change group	No-change group	p-value[a]
White blood cells (/μL)	14,300 ± 4600	15,700 ± 6900	0.30
Neutrophils (%)	65.9 ± 10.9	76.3 ± 12.7	<0.001
Hematocrit (%)	33.7 ± 2.9	34.1 ± 2.3	0.36
Platelet count ($\times 10^4$/μL)	36.2 ± 9.6	30.9 ± 9.9	0.001
Albumin (g/dL)	3.8 ± 0.4	3.6 ± 0.4	0.004
Aspartate aminotransferase (IU/L)	165 ± 424	155 ± 312	0.88
Alanine aminotransferase (IU/L)	122 ± 243	115 ± 255	0.82
Sodium (mEq/L)	134.9 ± 2.4	134.7 ± 2.5	0.61
Total bilirubin (mg/dL)	1.0 ± 0.9	1.0 ± 0.9	0.71
C-reactive protein (mg/dL)	6.7 ± 4.4	9.8 ± 5.8	<0.001
Ferritin (ng/mL)	158 ± 157	186 ± 117	0.22
Brain natriuretic peptide (pg/mL)	67.0 ± 81.2	76.7 ± 143.0	0.13
Left ventricular ejection fraction (%)	70.9 ± 6.5	70.3 ± 6.3	0.52

Values are presented as mean ± standard deviation.
[a]Student's t-test

Table 4. Multivariate regression analysis.

Factor	HR	p-value	95% CI
Age at BCG vaccination	1.004	0.50	0.99–1.02
Days after vaccination	0.995	<0.001	0.993–0.997
No. of principal symptoms	0.57	0.11	0.29–1.14
Neutrophil count	1.04	0.13	0.99–1.08
Platelet count	1.01	0.58	0.97–1.06
Albumin	2.76	0.13	0.73–10.73
C-reactive protein	0.94	0.25	0.84–1.05
Brain natriuretic peptide	0.995	0.07	0.990–1.001

BCG, Bacille Calmette–Guérin; CI, confidence interval; HR, hazard ratio.

significantly higher platelet counts ($p = 0.001$) and albumin levels ($p = 0.004$). No significant difference was observed in the left ventricular ejection fraction at diagnosis between the two groups.

The results of a multivariate analysis of demographic and severity variables are summarized in Table 4. The interval from vaccination to symptom onset was identified as being most closely positively associated with a BCG site change (hazard ratio, 0.995; $p < 0.001$; 95% confidence interval, 0.993–0.997). Other factors were not found to be significant.

Analysis of recurrent cases

As shown in Table 5, 9 of the 177 (5.1%) patients experienced recurrence of KD symptoms. The presence or absence of a BCG site change at recurrence in each patient is summarized in Table 5. Patients who experienced recurrence 32, 133, 433, and 730 days after BCG vaccination had a BCG site change. Two patients (Nos. 1 and 4) showed different patterns of BCG site changes between the first and second onset of symptoms; the change occurred 32 and 730 days after vaccination in patient Nos. 1 and 4, respectively.

Table 5. BCG inoculation site change in recurrent cases.

Patient No.	Sex	Age at vaccination (days)	1st onset			2nd onset		
			Days at onset	Days after vaccination	BCG change	Days at onset	Days after vaccination	BCG change
1	M	130	137	7	−	162	32	+
2	M	97	230	133	+	530	433	+
3	F	153	785	632	−	1080	927	−
4	F	127	857	730	+	1146	1019	−
5	M	112	1021	909	−	1084	972	−
6	M	130	1120	990	−	1832	1702	−
7	F	92	1343	1251	−	1618	1526	−
8	M	77	1765	1688	−	1863	1786	−

BCG, Bacille Calmette–Guérin; M, male; F, female.

Treatment profile and patients' prognosis

Resistance to high-dose gamma-globulin therapy (2 g/kg per dose) was observed in 26 of 83 (31%) patients in the change group and 35 of 94 (37%) patients in the no-change group; there was no significant difference between the groups. The incidence of coronary artery dilatation of at least +2.5 SD units (evaluated by the method reported by Kobayashi et al.[6]) was 17 of 83 (20%) patients in the change group and 21 of 94 (22%) patients in the no-change group; residual coronary artery aneurysms of at least 5 mm were not noted in either group, and the treatment and prognosis did not differ between the groups.

Discussion

After BCG vaccination, the patient's immunological status changes over time. BCG vaccination leads to the establishment of anti-infection immunity in about 4 weeks; this is followed by sustained systemic immune responses over 10 to 15 years, although inoculated BCG bacteria appear to remain at the inoculation site for only about 6 months.[7] A BCG site change in patients with KD is histologically characterized by marked infiltration of helper T cells and macrophages and expression of interleukin-1β tumor necrosis factor-α, interferon-γ, and interleukin-2, indicating a delayed hypersensitivity reaction.[8,9] Yokota et al.[10] investigated various BCG-derived proteins and found a protein that reacted with only sera collected during the convalescent stage of KD. They identified the protein to be 65-kDa heat shock protein (HSP65). In addition, a mitochondrial protein designated P1 (human homolog of bacterial HSP65) also reacted to the convalescent sera, showing cross-reactive responses to both mycobacterial HSP65 and P1 antigen among patients with KD. Furthermore, Sireci et al.[11] showed that a significant fraction of CD4 and CD8 T-cell clones from patients with KD recognized the epitopes on mycobacterial HSP65 and human HSP63 (P1 antigen), suggesting a possible pathogenic role of HSP-specific T cells in KD. These findings suggest that the cause of hypersensitivity reactions to BCG in patients with KD can be attributed to cross-reactivity between mycobacterial HSP65 and its human homolog. Therefore, the most important contributor to the development of a BCG site change is whether the condition of the immune

system allows delayed hypersensitivity reactions to occur at the site.

In the present study, a BCG site change was observed in patients who experienced the onset of KD symptoms from 31 to 806 days after BCG vaccination, but not in those who experienced onset outside this interval. There was a clear difference in the prevalence of a BCG site change before and after 30 days after vaccination. This observation can be interpreted as follows in relation to delayed hypersensitivity: the anti-infection immunity acquired by BCG vaccination is associated with the secondary antigens responsible for the onset of KD symptoms. During the first 30 days after BCG vaccination, the BCG-sensitized T-lymphocyte population has not grown sufficiently to cause cross-reaction with KD-related antigens. However, local immune responses diminish after a long time has elapsed after BCG vaccination, such that responses against KD-related antigens disappear in about 800 days.

A nationwide survey conducted in Japan from 2005 to 2006 identified a BCG site change in 49.9% of patients with KD, with a particularly high prevalence of $\geq$70% in children aged 3 to 20 months, demonstrating an age-related difference in the prevalence of BCG site change.[3] Similar results have also been reported elsewhere.[5] However, some of the infants with KD symptoms had a BCG site change while others did not because they had received BCG vaccination at different ages. These age-related differences made it difficult to show a clear difference in the prevalence of a BCG site change before and after 30 days following vaccination, as observed in the present study. The histological findings and immunological mechanisms of a BCG site change also support the appropriateness of considering differences according to the interval from vaccination instead of according to age.

Notably, 21% of the patients with symptom onset from 31 to 806 days after BCG vaccination had no BCG site change. Local reactions to BCG vaccination are known to vary among infants depending on immunization techniques and various other factors. Such differing patterns of immune acquisition following vaccination might lead to varying patterns of cross-reaction with KD antigens, although this possibility was not addressed in the present study. In terms of severity of symptoms, no significant intergroup difference was observed in the number of patients with a confirmed diagnosis, while the number of principal symptoms was significantly lower in the change group (which had a higher number of younger patients). This is likely to be attributed to the known lower prevalence of cervical lymphadenopathy in younger patients with KD.[12] With respect to the severity of abnormal examination results, the presence of strong acute inflammation was suggested in the no-change group. Lai et al.[5] reported increased white blood cell and platelet counts and increased intensity of inflammation in patients with a BCG site change, which is inconsistent with the findings of the present study. In contrast, a Japanese nationwide survey of patients with KD performed from 1997 to 1998 showed decreased neutrophil counts, decreased CRP levels, and increased platelet counts in younger patients.[13] Therefore, considering the effect of age, we conducted a multivariate analysis including the interval from vaccination to onset (approximately the age at onset) and the severity of symptoms and abnormal examination results, and we found that a BCG site change was more closely associated with the interval from vaccination than with the severity of inflammation.

KD is common in Japan and other parts of East Asia, and its prevalence is considered to reflect the genetic background of the relevant ethnic groups. The sibling risk

ratio is 1.5%, and a high percentage (0.89%) of parents of patients with KD have a history of KD. Differences in genetic susceptibility are thus considered to exist with the same ethnic group. In contrast, a susceptibility gene for KD has been reported, and genetic factors are associated with the onset of KD or the manifestation of KD symptoms in carriers. Genetic predisposing factors in the host are thought to be associated with the onset or development of KD symptoms.[14] We therefore investigated whether any changes in these factors occurred at the first and second onset of KD symptoms within individual hosts. Two of the three patients with a BCG site change (patient Nos. 1 and 4) showed different patterns of development of their BCG site change at the first and second onset of KD symptoms depending on the interval from vaccination; that is, only symptom onset from 31 to 806 days after vaccination was associated with a BCG site change. This was consistent with the relationship between a BCG site change and the interval from vaccination to onset. The present results suggest that the interval from vaccination is more pivotal to the development of a BCG site change than other factors, such as host-related factors.

Various potential factors have been identified as being associated with the development of a BCG site change in patients with KD. Of these factors, the interval from BCG vaccination to symptom onset was found to be the most relevant in the present study. However, this was a single-center retrospective study, and further studies are needed to verify our results. Analyses of interleukin-2 and other cytokines were possible for only a subset of patients in this study, and the results are accordingly not included in this report. Although this study could not determine the exact mechanism by which a BCG site change occurs, these results may contribute to elucidating the pathogenesis of BCG site changes and the onset of KD symptoms in future studies.

Conclusions

A BCG inoculation site change in patients with KD was more closely associated with the interval from vaccination to onset than with the severity of symptoms, abnormal examination results, or host genetic predisposing factors. A BCG site change occurred only in patients with an onset of KD symptoms from approximately 30 to 800 days after vaccination. During this period, delayed hypersensitivity reactions between host anti-infection immunity acquired by the BCG vaccination and secondary antigens involved in the onset of KD symptoms might have been induced locally at the site of the BCG scar.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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