these 59 genes formed distinctive groupings in terms of correlation across the 216 genes. Namely, up- or down-regulated genes (black circles in Fig. 4C) localized outside the diagonal bundles of dots (purple and crimson circles in Fig. 4C) over a wide range of intensities. In contrast, these 59 genes showed no obvious correlation patterns in other patient samples (data not shown).

Notably, the aGp array has additional practical advantages for clinical use because the obtained data were found to be very stable after scanning, even without sealing or other direct countermeasures against exposure to ozone (Supplementary Fig. S3). Moreover, the background level of the data after 19–27 days were largely reduced compared with 1 day data, which confirmed the reliability of the data even with low signal intensity. Taken together, these results indicate that aGp arrays are clinically useful because they can clearly distinguish TA patient profiles from other patients by the Spearman rank correlation using all 216 probes.

3.5. *Comparison between aGp array, Agilent's microarray and qPCR*

Next, the performance of aGp arrays was compared with other tools to examine the variation of the expression patterns of the genes in the 13 TA patient samples. First, all TA samples (TA1–13) were applied to Agilent's Whole Human Genome Microarray (4 × 44K). DEFA3, interleukin (IL)-4 and IL-10 were selected for further analysis because they are known to be highly associated with various autoimmune diseases.[14] When the expression profiles of each gene from aGp array, Agilent's microarray and qPCR are compared, represented by green, purple and blue lines, respectively, it is apparent that DEFA3 achieved almost complete correlation among the three measurement systems (Fig. 5A). This correlation is surprising considering the distinct sequences of the probes used in each measurement and further confirmed the reliability of the data obtained by aGp arrays. In contrast, IL-4 and IL-10 showed distinct expression patterns depending on the system (Fig. 5Bi and ii).

Notably, IL-4 and IL-10 were expressed at remarkably low levels compared with DEFA3 (indicated by arrows in Fig. 6A). The scatter plot of log signal intensities from the HV pool (horizontal) and patient TA8 (vertical) show that the signal intensities of IL-4 and IL-10 are considerably lower than that of DEFA3. In addition, the correlation coefficients of DEFA3 for the averaged signal intensities of the aGp array and Agilent's microarray against qPCR normalized to GAPDH (0.93 and 0.96) are significantly higher than those of IL-4 (−0.52 and 0.52) and IL-10 (0.12 and −0.06), respectively (Fig. 6B).

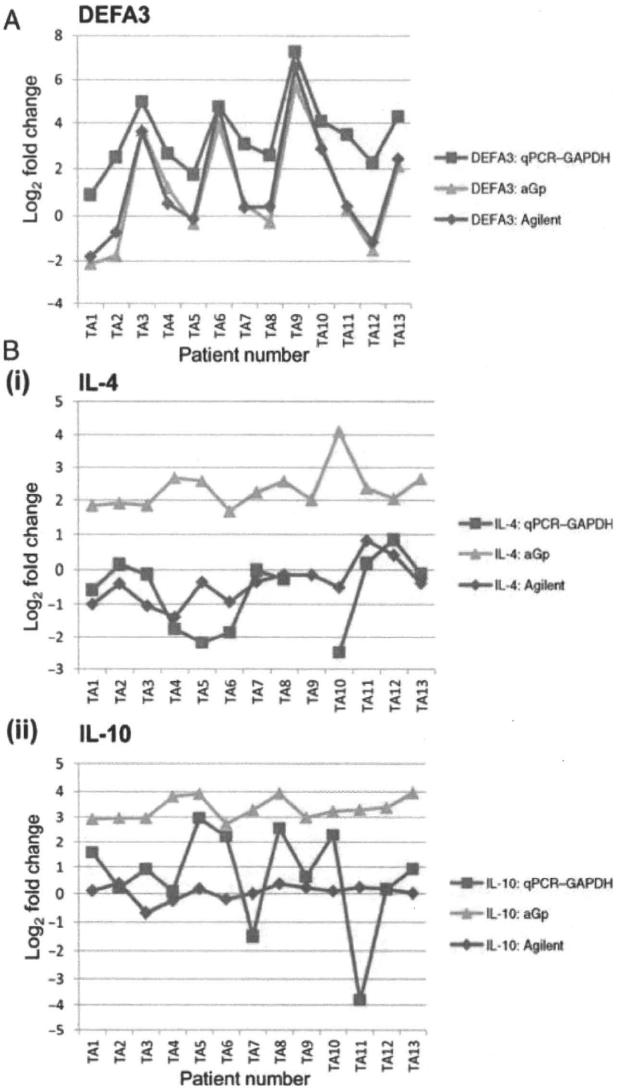
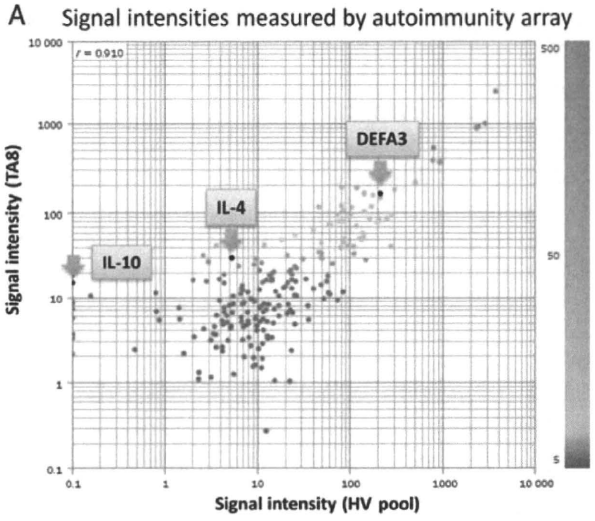


Figure 5. Expression profiles of DEFA3 (A), IL-4 (Bi) and IL-10 (Bii) in the 13 TA patients. The vertical axis indicates the $\log_2$ ratio measured by qPCR–GAPDH (blue), aGp array (green) and Agilent's microarray (purple). The horizontal axis indicates the patient number. (A) The comparison of the values of peaks and valleys for DEFA3 revealed that these three methods yielded very similar expression profiles. (Bi and ii) The expression profiles of the IL-4 and IL-10 genes for the 13 TA patients were different between qPCR–GAPDH (blue), aGp array (green) and Agilent's microarray (purple).

3.6. *Comparison with nCounter*TM

To investigate whether aGp or Agilent provided more reliable results, we employed nCounter™ by NanoString[15] and measured the expression levels of 25 relevant genes (aGps) using one patient sample (TA8). The raw data revealed that the expression levels of IL-4 and IL-10 in this patient were very low

A



B

	Probe	Correlation coefficient	Average of signals	
			HV pool	TAs
aGp	IL-4	-0.52	5.10	29.00
	IL-10	0.12	-0.76	10.53
	DEFA3	0.93	199.45	1534.39
Agilent	IL-4	0.52	17.15	13.55
	IL-10	-0.06	17.65	18.36
	DEFA3	0.96	58 208.93	277 417.99

Figure 6. The aGp array provides sufficient sensitivity for diagnostic application. (A) A scatter plot of the log of signal intensity of the HV pool versus patient TA8 is plotted along the horizontal and vertical axes, respectively. DEFA3, IL-4 and IL-10 genes are indicated by orange arrows. The attached colour bar indicates a relative intensity scale. (B) The correlation coefficients of the Genopal™ autoimmunity array and Agilent's microarray against qPCR are shown. The correlation coefficient of DEFA3 for both Genopal™ and Agilent's arrays was high. On the other hand, because the signal intensities of IL-4 and IL-10 were within two orders of magnitude, as shown in the columns on the right, the correlation coefficient of IL-4 or IL-10 is lower than DEFA3.

and that 10 of the 25 genes were up-regulated more than 2-fold compared with the HV sample (green arrows in Supplementary Fig. S4). Comparison of the relative induction rates between aGp, Agilent and nCounter™ revealed that 12 of the 25 genes (yellow arrows in Fig. 7) had similar results in all three methods, whereas nCounter™ favoured aGp arrays (turquoise arrows) or Agilent (pink arrows) for three and the six genes, respectively. The other four genes (purple arrows) showed distinct results among these three methods. As both nCounter™ and qPCR favoured aGp arrays for IL-10 and Agilent for IL-4 (Supplementary Fig. S5), it is difficult to determine at present which microarray provided more reliable results. Taken together, these results led us to conclude that aGp arrays provide reliable data at a level similar to Agilent's microarray, particularly when the expression levels of the target gene are not very low.

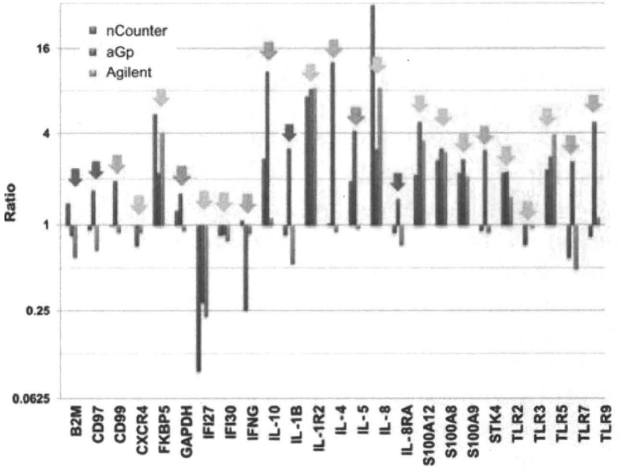


Figure 7. Comparison of the mRNA expression levels (y-axis) of 25 selected genes (x-axis) in PBMCs of patient TA8 using nCounter™ (blue bars), aGp array (Genopal™; red bars) or Agilent's microarray (green bars). Yellow arrows indicate genes that had similar expression levels in the three methods. Turquoise or pink arrows indicate the genes used by nCounter™ to favour aGp or Agilent in terms of their expression levels. Purple arrows indicate the genes that exhibited different expression levels among the three methods.

4. Discussion

In the present study, we describe a novel platform termed Genopal™ that is ideal for the preparation of focused microarray (Fig. 1). We first designed the iGp array, examined its performance using Cy5-labelled synthetic oligonucleotides as hybridization probes (Fig. 2A) and determined that it yielded highly reproducible data (Fig. 2B; CV was 10.6% on average). We also prepared the aGp array and tested their clinical applicability using RNA samples from a small volume of PBMCs obtained from TA patients (Fig. 3B and Supplementary Table S3). We do not exclude the usefulness of other genes that were not installed into the aGp array. Nonetheless, we showed that the aGp array is able to distinguish TA patient profiles from HVs and patients with other autoimmune diseases based on the Spearman rank correlation using all 216 probes (Fig. 4). Notably, this data-processing protocol is quite simple and extensible; all that is needed is to calculate the ratio of the Spearman rank correlation for the patients' and HV pool samples. This protocol is useful only for analysis of small number of genes and cannot be applied to conventional genome-wide microarrays, which is another advantageous point of Genopal™. Comparison of the data obtained by an aGp array, Agilent's microarray, qPCR and nCounter™ indicated that these methods exhibited similar expression profiles with regard to target genes with high signal intensities (Figs 5–7).

Using aGp arrays, we also identified here over 10 genes that were up- or down-regulated in almost all TA patients tested (Supplementary Table S4). Among the up-regulated genes, S100A8, S100A9 and S10012 are inflammation-related genes. This is not unexpected because TA is an autoimmune disease that involves enhanced inflammation of the aorta. The up-regulation of amphiregulin (AREG), which is a member of the epidermal growth factor family, may be related to the ventricular hypertrophy often observed in TA patients. Alternatively, it may be involved in the maintenance of inflammatory situations by inducing the proliferation of regulatory cells in these phenomena. Enhanced expression of IL-1 receptor, type II (IL-1R2), a decoy receptor that traps pro-inflammatory IL-1β and does not initiate subsequent signalling events, may be due to a negative feedback response to the hyperactive inflammation in the aorta of TA patients (which results partially from steroid therapy). Defensins, which include DEFA3, are a family of microbicidal and cytotoxic peptides that are abundant in the granules of neutrophils and are thought to be involved in host Defence, which suggest the involvement of infection in the pathogenesis of TA. In contrast, interferon gamma (IFN-G) and interferon-induced genes (G1P2, IFI44, IFIT1, IFIT2 and IFI27) were down-regulated in many TA patients, which distinguish TA from SLE and ITP, as these genes are up-regulated in the latter autoimmune diseases. As there is no reliable diagnostic tool for TA at present, these genes may also be useful as diagnostic markers of the disease when used in combination with the Spearman rank correlation method described above.

One of the practical applications of iGp and aGp arrays would be their use in the diagnosis of patients afflicted with fever of unknown origin (FUO). Our proposal is presented schematically in Supplementary Fig. S6. Namely, a 2-ml blood sample may be collected for every new FUO patient using PAXgene Blood RNA Kits of Quiagen, total RNA prepared, labelled with Cy5 and applied to an aGp array. As the aGp array harbours genes that are either up- or down-regulated in the PBMCs of several autoimmune diseases, such as SLE,[11] ITP,[11] RA[12] and/or vasculitis,[13] we expect to observe positive signals if the patient's FUO is related to any of these autoimmune diseases. In this case, the FUO patient may be directly referred to an autoimmune disease specialist to receive appropriate care. If the screen yielded negative results, other sample sources, such as phlegm and/or excrement, depending on the dubious infectants, may be collected for RNA preparation and screening using the iGp array. If a positive signal is detected, the patients could then be treated appropriately at the bedside. Recent reports indicate that GenopalTM is useful not only for cRNA but also for miRNA samples.[16–20]

Taken together, we conclude that GenopalTM is an advantageous platform for focused microarrays with regard to its low cost, reliability, ease of storage, speed and suitability for large-scale production. The data-processing protocol we developed here might be quite useful for the diseases that lack diagnostic tools using conventional clinical methods.

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Supplementary Data: Supplementary Data are available at www.dnaresearch.oxfordjournals.org.

Authors' contributions

D.O. and T.F. designed and performed research, T.T. performed research on the infectant array, T.I. and S.K. provided TA patient samples and monitored the results, T.A. developed the GenopalTM system and H.N. designed the research and wrote the manuscript. K.Y. provided BD samples.

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Evaluation of the Chronic Kidney Disease Epidemiology Collaboration equation for estimating the glomerular filtration rate in multiple ethnicities

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An equation from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) provides more accurate estimates of the glomerular filtration rate (eGFR) than that from the modification of diet in renal disease (MDRD) Study, although both include a two-level variable for race (Black and White and other). Since creatinine generation differs among ethnic groups, it is possible that a multilevel ethnic variable would allow more accurate estimates across all groups. To evaluate this, we developed an equation to calculate eGFR that includes a four-level race variable (Black, Asian, Native American and Hispanic, and White and other) using a database of 8254 patients pooled from 10 studies. This equation was then validated in 4014 patients using 17 additional studies from the United States and Europe (validation database), and in 1022 patients from China (675), Japan (248), and South Africa (99). Coefficients for the Black, Asian, and Native American and Hispanic groups resulted in 15, 5, and 1% higher levels of eGFR, respectively, compared with the White and other group. In the validation database, the two-level race equation had minimal bias in Black, Native American and Hispanic, and White and other cohorts. The four-level ethnicity equation significantly improved bias in Asians of the validation data set and in Chinese. Both equations had a large bias in Japanese and South African patients. Thus, heterogeneity in performance among the ethnic and geographic groups precludes use of the four-level race equation. The CKD-EPI two-level race equation can be used in the United States and Europe across a wide range of ethnicity.

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KEYWORDS: creatinine; ethnicity; glomerular filtration rate

Chronic kidney disease (CKD) is a worldwide health problem, affecting all racial and ethnic groups that have been investigated.[1] In the United States, chronic kidney failure disproportionately burdens racial and ethnic minorities. Incidence rates for chronic kidney failure treated by dialysis and transplantation are 3.6 and 1.4 times higher in Blacks and Asians, respectively, compared with Whites, and 1.5 times higher in Hispanics compared with non-Hispanics.[2] Outside of the United States, Taiwan and Japan have the highest prevalence rates of treated kidney failure.[2,3] Data on the prevalence, etiology, and outcomes of earlier stages of kidney disease in these groups are likely to be inaccurate due, at least in part, to the lack of accurate glomerular filtration rate (GFR) estimates.

The Modification of Diet in Renal Disease (MDRD) Study equation utilizes a two-level variable for race (Black vs White and other). The coefficient for Blacks leads to higher values for estimated GFR (eGFR) compared with Whites for the same level of creatinine, because of differences between Blacks vs Whites in factors other than GFR that affect the serum level of creatinine (non-GFR determinants), especially higher creatinine generation from muscle and diet.[4,5] It is widely believed that there are also differences in creatinine generation in other racial, ethnic, and geographic groups, which are not captured by current equations.[6,7] Consistent with this assumption, introduction of coefficients for use in the MDRD Study equation in China and Japan improves its performance in these populations.[8,9]

We recently reported a new equation, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation,

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based on creatinine, age, sex, and a two-level variable for race, which is more accurate than the MDRD Study equation, particularly at higher levels of GFR and in populations without CKD,[5,10,11] and provides better risk prediction.[12,13] We hypothesized that the performance of the CKD-EPI equation could be further improved in Asians and in Native Americans and Hispanics by utilizing coefficients specific for these groups. In this study, we report on the development of an GFR-estimating equation that includes a four-level race variable in a diverse population from the United States and Europe, and its evaluation compared with the CKD-EPI (two-level race) equation in separate populations from the United States and Europe as well as in populations from other countries.

RESULTS

The clinical characteristics differed significantly among racial and ethnic groups. In the development data set (Table 1a), mean measured GFR ranged from 55 to 73 ml/min per 1.73 m^2 among racial/ethnic groups, and was lower in Blacks and Asians and higher in Native Americans and Hispanics compared with Whites and others. Blacks were older, more likely to be female, and had a larger body size compared with the other groups. In the CKD-EPI external validation data set, measured GFR ranged from 53 to 105 ml/min per 1.73 m^2 and was lower in Asians and higher in Native Americans and Hispanics compared with Whites and others (Table 1b). In the non-US and Europe validation data set, measured GFR ranged from 53 and 60 ml/min per 1.73 m^2, and body mass index (BMI) was lower than in the CKD-EPI development and validation data sets (Table 1b). Supplementary Appendix A and B describe the distribution of race and ethnic groups for each study.

Table 2 shows the coefficients for each race and ethnic groups refit in the CKD-EPI combined development and internal validation data set. The coefficients for Black and Asian are significantly larger than the reference group (White and other), resulting in higher eGFR for the same level of creatinine. The coefficient for Native American and Hispanic was smaller and not statistically significant, but was retained in the model. For both the two- and four-level race equations, eGFR is 15% higher for Blacks than for Whites or others. In the four-level race equation, eGFR is 5% higher in Asians but only 1% higher in Native Americans and Hispanics compared with Whites or others. Table 3 shows the two- and four-level race equations developed using the coefficients from the combined development and internal validation data sets, expressed for different combinations of race, sex, and serum creatinine.

Tables 4 and 5 show the performance of both models in the two external validation data sets. In the CKD-EPI

Table 1a | Clinical characteristics of the participants in development data sets

Variable	Overall	Race/ethnicity				*P*-values
		White and other	Black	Asian	Native American and Hispanic	
N	8254	5216	2585	100	353	
Age, mean (s.d.) in years	47 (15)	44 (15)	53 (12)	49 (15)	43 (12)	<0.001
Age categories, N *(%)*						<0.001
<40 years	3076 (37)	2464 (47)	422 (16)	36 (36)	154 (44)	
40–65 years	4154 (50)	2149 (41)	1766 (68)	50 (50)	189 (54)	
>65 years	1024 (12)	603 (11)	397 (16)	14 (11)	10 (3)	
Sex, N *(%)*						<0.001
Female	3606 (44)	2353 (45)	1019 (39)	41 (41)	193 (55)	
Male	4648 (56)	2863 (55)	1566 (61)	59 (59)	160 (45)	
Diabetes, N *(%)*						<0.001
Yes	2406 (29)	1885 (36)	280 (11)	33 (33)	208 (59)	
No	5848 (71)	3331 (64)	2305 (89)	67 (67)	145 (41)	
Transplant, N *(%)*						<0.001
Yes	360 (4)	330 (6)	24 (1)	5 (5)	1 (0.3)	
No	7894 (96)	4886 (94)	2561 (99)	95 (95)	352 (100)	
GFR mean (s.d.), ml/min per 1.73 m^2	68 (40)	73 (43)	55 (27)	57 (31)	90 (45)	<0.001
Serum creatinine, mean (s.d.), mg/dl	1.66 (1.16)	1.58 (1.19)	1.87 (1.09)	1.73 (0.91)	1.23 (1.02)	<0.001
Body surface area, mean (s.d.), m^2	1.91 (0.24)	1.90 (0.23)	2.00 (0.25)	1.77 (0.21)	1.91 (0.25)	<0.001
BMI, mean (s.d.), kg/m^2	28 (6)	27 (5)	31 (7)	26 (5)	31 (9)	<0.001
BMI categories, N *(%)*						<0.001
<20 kg/m^2	287 (3)	218 (4)	60 (2)	4 (4)	5 (1)	
20–25 kg/m^2	2447 (30)	1896 (36)	446 (17)	40 (40)	65 (18)	
26–30 kg/m^2	2922 (35)	1930 (37)	857 (33)	37 (37)	98 (28)	
>30 kg/m^2	2598 (31)	1172 (23)	1222 (47)	19 (19)	185 (52)	

Abbreviations: BMI, body mass index; GFR, glomerular filtration rate.
To convert GFR from ml/min per 1.73 m^2 to ml/s per 1.73 m^2, multiply by 0.0167.

Table 1b | Clinical characteristics of the participants in validation data sets

	CKD-EPI (US and Europe)				Non-US and Europe			
Variable	White and other	Black	Asian	Native American and Hispanic	Asian	Asian	Black	*P*-values
N	3378	384	67	185	248	675	99	
Age, mean (s.d.) in years	49 (15)	50 (15)	51 (15)	45 (12)	50 (18)	50 (15)	47 (17)	0.001
Age categories, N *(%)*								<0.001
<40 years	978 (29)	112 (29)	19 (28)	68 (37)	95 (38)	207 (31)	42 (43)	
40–65 years	1898 (56)	224 (58)	35 (52)	107 (58)	92 (37)	333 (49)	42 (43)	
>65 years	502 (15)	48 (13)	13 (19)	10 (5)	61 (25)	135 (20)	15 (15)	
Sex, N *(%)*								0.001
Female	1513 (45)	184 (48)	32 (48)	130 (70)	112 (45)	328 (49)	49 (49)	
Male	1865 (55)	200 (52)	35 (52)	55 (30)	136 (55)	347 (51)	50 (50)	
Diabetes, N *(%)*								<0.001
Yes	975 (29)	95 (25)	14 (21)	119 (64)	35(14)	21(3)	6 (6)	
No	2403 (71)	289 (75)	53 (79)	66 (67)	213 (86)	654 (97)	93 (94)	
Transplant, N *(%)*								<0.001
Yes	1072 (32)	52 (14)	7 (10)	3 (2)	0	0	0	
No	2306 (68)	332 (86)	60 (90)	182 (98)	0	0	0	
GFR, mean (s.d.), ml/min per 1.73 m^2	69 (36)	62 (34)	53 (31)	105 (47)	53 (31)	55 (35)	61 (32)	<0.001
Serum creatinine, mean (s.d.), mg/dl	1.48 (0.94)	1.80 (0.29)	1.99 (1.41)	0.90 (0.73)	1.24 (0.56)	2.25 (2.18)	1.77 (1.71)	<0.001
Body surface area, mean (s.d.), m^2	1.90 (0.23)	1.95 (0.23)	1.70 (0.20)	1.98 (0.29)	1.62 (0.18)	1.71 (0.18)	1.77 (0.17)	<0.001
BMI, mean (s.d.), kg/m^2	27 (5)	30 (7)	24 (4)	34 (8)	23 (4)	24 (4)	26 (5)	<0.001
BMI categories, N *(%)*								<0.001
<20 kg/m^2	225 (7)	17 (4)	5 (7)	2 (1)	55 (22)	107 (16)	15 (15)	
20–25 kg/m^2	1223 (36)	84 (22)	34 (51)	22 (12)	137 (55)	354 (52)	44 (44)	
25–30 kg/m^2	1178 (35)	115 (30)	24 (36)	49 (26)	45(18)	181 (27)	20 (20)	
>30 kg/m^2	752 (22)	168 (44)	4 (6)	112 (61)	11 (4)	33 (5)	20 (20)	

Abbreviations: BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; GFR, glomerular filtration rate.
To convert GFR from ml/min per 1.73 m^2 to ml/s per m^2, multiply by 0.0167.

Table 2 | Race/ethnicity coefficients (95% confidence intervals)[a]

Equation	White and other	Black	Asian	Native American and Hispanic
Two-level race	1.0 (reference group)	1.157 (1.144, 1.170)	1.0	1.0
Four-level race	1.0 (reference group)	1.160 (1.146, 1.173)	1.052 (1.004, 1.102)	1.010 (0.984, 1.037)

Coefficients are adjusted for creatinine, sex, and age.
[a]Corresponds to percent increase in estimated glomerular filtration rate (eGFR) for the same level of serum creatinine.

validation data set, performance of the equation with the two- and four-level race terms was similar in both the Black and White and other groups (Table 4). In Asians, there was a significant improvement in bias and root mean square error with the four-level compared with the two-level equation (0.8 (−2.2, 2.6) ml/min per 1.73 m^2 vs 2.1 (0.3, 4.4) ml/min per 1.73 m^2 ($P<0.005$) and 0.293 (0.178, 0.424) vs 0.302 (0.188, 0.436), $P=0.003$)), but there was a small higher interquartile range with the four-level equations (12.3 (9.0, 16.1) vs 10.5 (8.0, 14.6) ml/min per 1.73 m^2 ($P=0.001$)) and no significant difference in percentage of estimates within 30% of the measured GFR (P_{30}). There were no significant differences in performance between the two equations for Native Americans and Hispanics. In the Chinese data set (Table 5, column 1), as in the Asians in the CKD-EPI validation data set, there was an improvement in performance with the four-level race equation compared with the two-level race equation in bias (1.3 (0.6, 2.2) vs 2.7 (1.9, 3.7) ml/min per 1.73 m^2 ($P<0.0001$)), interquartile range (15.5 (14.4, 17.4) vs 16.7 (15.0, 18.5) ml/min per 1.73 m^2, $P<0.0001$), root mean square error (0.318 (0.295, 0.343) vs 0.325 (0.302, 0.348) ml/min per 1.73 m^2, $P=0.002$)), as well as in P_{30} (72.1 (68.7, 75.7) vs 73.2 (69.9, 76.6), $P=0.01$). In the Japanese data set (Table 5, column 2), performance for the two-level race equation was substantially worse than for the Asians in the CKD-EPI validation data set and not improved with the use of the four-level race equation. In the South African data set (Table 5, column 3), performance of both the two- and four-level race equations was substantially worse than for the Blacks in the CKD-EPI validation data set. Performance was better for the South African data set when the Black coefficient was not used (bias of −12.4 (−18.3, −7.6) with the use of the Black term vs −4.9 (−7.0, −0.5) ml/min per 1.73 m^2 without the use of the Black term).

Figure 1 summarizes the comparison of bias between the two- and four-level race equation by level of eGFR within

Table 3 | CKD-EPI equation for estimating GFR on the natural scale expressed for race, sex, and range of serum creatinine

Race	Sex	Serum creatinine	eGFR (ml/min per 1.73 m^2)
Two-level race equation			
Black	Female	≤0.7 mg/dl	$166 \times (0.993)^{Age} \times (Scr/0.7)^{-0.329}$
Black	Female	>0.7 mg/dl	$166 \times (0.993)^{Age} \times (Scr/0.7)^{-1.209}$
Black	Male	≤0.9 mg/dl	$163 \times (0.993)^{Age} \times (Scr/0.9)^{-0.411}$
Black	Male	>0.9 mg/dl	$163 \times (0.993)^{Age} \times (Scr/0.9)^{-1.209}$
White and other	Female	≤0.7 mg/dl	$144 \times (0.993)^{Age} \times (Scr/0.7)^{-0.329}$
White and other	Female	>0.7 mg/dl	$144 \times (0.993)^{Age} \times (Scr/0.7)^{-1.209}$
White and other	Male	≤0.9 mg/dl	$141 \times (0.993)^{Age} \times (Scr/0.9)^{-0.411}$
White and other	Male	>0.9 mg/dl	$141 \times (0.993)^{Age} \times (Scr/0.9)^{-1.209}$
Four-level race equation			
Black	Female	≤0.7	$167 \times (0.993)^{Age} \times (Scr/0.7)^{-0.328}$
Black	Female	>0.7	$167 \times (0.993)^{Age} \times (Scr/0.7)^{-1.210}$
Black	Male	≤0.9	$164 \times (0.993)^{Age} \times (Scr/0.9)^{-0.412}$
Black	Male	>0.9	$164 \times (0.993)^{Age} \times (Scr/0.9)^{-1.210}$
Asian	Female	≤0.7	$151 \times (0.993)^{Age} \times (Scr/0.7)^{-0.328}$
Asian	Female	>0.7	$151 \times (0.993)^{Age} \times (Scr/0.7)^{-1.210}$
Asian	Male	≤0.9	$149 \times (0.993)^{Age} \times (Scr/0.9)^{-0.412}$
Asian	Male	>0.9	$149 \times (0.993)^{Age} \times (Scr/0.9)^{-1.210}$
Hispanic and Native American	Female	≤0.7	$145 \times (0.993)^{Age} \times (Scr/0.7)^{-0.328}$
Hispanic and Native American	Female	>0.7	$145 \times (0.993)^{Age} \times (Scr/0.7)^{-1.210}$
Hispanic and Native American	Male	≤0.9	$143 \times (0.993)^{Age} \times (Scr/0.9)^{-0.412}$
Hispanic and Native American	Male	>0.9	$143 \times (0.993)^{Age} \times (Scr/0.9)^{-1.210}$
White and other	Female	≤0.7	$144 \times (0.993)^{Age} \times (Scr/0.7)^{-0.328}$
White and other	Female	>0.7	$144 \times (0.993)^{Age} \times (Scr/0.7)^{-1.210}$
White and other	Male	≤0.9	$141 \times (0.993)^{Age} \times (Scr/0.9)^{-0.412}$
White and other	Male	>0.9	$141 \times (0.993)^{Age} \times (Scr/0.9)^{-1.210}$

Abbreviations: CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; GFR, glomerular filtration rate.
To convert GFR from ml/min per 1.73 m^2 to ml/s per 1.73 m^2, multiply by 0.0167. To convert serum creatinine from mg/dl to μmol/l, multiply by 88.4. CKD-EPI equation coefficients derived from pooled development and internal validation data sets.
CKD-EPI two-level race equation expressed as a single equation: $GFR=141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] × 1.159 [if black] where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.
The four-level equation expressed as a single equation: $GFR=141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.210} \times 0.993^{Age} \times 0.993$ [if female] × 1.16 [if Black] × 1.05 [if Asian] × 1.01 [if Hispanic and Native American] where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is −0.328 for females and −0.412 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ.
In the table, the multiplication factors for race and sex are incorporated into the intercept, resulting in different intercepts for age and sex combinations.

Table 4 | Performance in CKD-EPI external validation data set (US and Europe) by race/ethnicity

Measures	Equation	Total	White and other	Black	Asian	Native American and Hispanic
N		4014	3378	384	67	185
Bias, ml/min per 1.73 m^2	Two-level	2.5 (2.1, 2.9)	2.8 (2.4, 3.2)	−0.8 (−2.0, 0.6)	2.1 (0.3, 4.4)	2.3 (−2.1, 5.1)
	Four-level	2.5 (2.1, 2.9)	2.9 (2.5, 3.4)	−0.9 (−2.0, 0.6)	0.8 (−2.2, 2.6)	1.6 (−3.0, 4.2)
IQR, ml/min per 1.73 m^2	Two-level	17.0 (16.1, 17.6)	16.8 (16.0, 17.6)	15.1 (12.6, 17.6)	10.5 (8.0, 14.6)	25.6 (20.8, 32.0)
	Four-level	17.0 (16.2, 17.6)	16.8 (16.0, 17.6)	15.1 (12.6, 17.6)	12.3 (9.0, 16.1)	26.1 (20.8, 32.2)
P_{30}, %	Two-level	84 (83, 85)	84 (83, 86)	82 (78, 85)	85 (76, 93)	80 (74, 85)
	Four-level	84 (83, 85)	84 (83, 85)	82 (80, 85)	85 (76, 93)	81 (76, 87)
RMSE	Two-level	0.250 (0.242, 0.259)	0.250 (0.240, 0.258)	0.242 (0.221, 0.265)	0.302 (0.188, 0.436)	0.265 (0.223, 0.310)
	Four-level	0.250 (0.242, 0.259)	0.250 (0.240, 0.259)	0.243 (0.221, 0.266)	0.293 (0.178, 0.424)	0.264 (0.222, 0.310)

Abbreviations: CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; GFR, glomerular filtration rate; IQR, interquartile range, 25–75th percentile; P_{30}, percentage of GFR estimates within 30% of measured GFR; RMSE, root mean square error.
Bias is calculated as measured GFR−estimated GFR.
Numbers in brackets are 95% confidence intervals.
To convert GFR from ml/min per 1.73 m^2 to ml/s per 1.73 m^2, multiply by 0.0167.

each racial/ethnic category. In the CKD-EPI validation data set, using either the two- and four-level race equation, bias was less than ~5 ml/min per 1.73 m^2 except for Blacks with eGFR >90 ml/min per 1.73 m^2, as we have previously reported. In the Asians in the CKD-EPI data set and in the Chinese data sets, the bias exceeded 5 ml/min per 1.73 m^2 for some eGFR groups, but improved with the use of the four-level race equation. For both equations, the bias varied substantially throughout the eGFR range in the Japanese and South African data sets.

DISCUSSION

Differences across race and ethnic groups in relationships between serum creatinine and measured GFR primarily

Table 5 | Performance in non-US and Europe external validation data set by country and race/ethnicity

Measures	Equation	China (Asian)	Japan (Asian)	South Africa (Black)
N		675	248	99
Bias, ml/min per 1.73 m^2	Two-level	2.7 (1.9, 3.7)	−17.8 (−20.1, −14.7)	−12.4 (−18.3, −7.6)
	Four-level	1.3 (0.6, 2.2)	−21.4 (−23.3, −18.2)	−12.5 (−18.3, −7.6)
IQR, ml/min per 1.73 m^2	Two-level	16.7 (15.0, 18.5)	21.0 (18.5, 23.9)	28.0 (20.8, 33.3)
	Four-level	15.5 (14.4, 17.4)	23.5 (20.4, 26.0)	28.0 (20.8, 33.4)
P_{30}, %	Two-level	73.2 (69.9, 76.6)	29.4 (23.8, 35.1)	55.6 (46.5, 64.6)
	Four-level	72.1 (68.7, 75.7)	36.3 (30.6, 42.3)	55.6 (46.5, 64.6)
RMSE	Two-level	0.325 (0.302, 0.348)	0.469 (0.424, 0.515)	0.326 (0.292, 0.361)
	Four-level	0.318 (0.295, 0.343)	0.507 (0.463, 0.553)	0.327 (0.292, 0.362)

Abbreviations: CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; GFR, glomerular filtration rate; IQR, interquartile range, 25–75th percentile; P_{30}, percentage of GFR estimates within 30% of measured GFR; RMSE, root mean square error.
Bias is calculated as measured GFR−estimated GFR.
Numbers in brackets are 95% confidence intervals.
To convert GFR from ml/min per 1.73 m^2 to ml/s per 1.73 m^2, multiply by 0.0167.

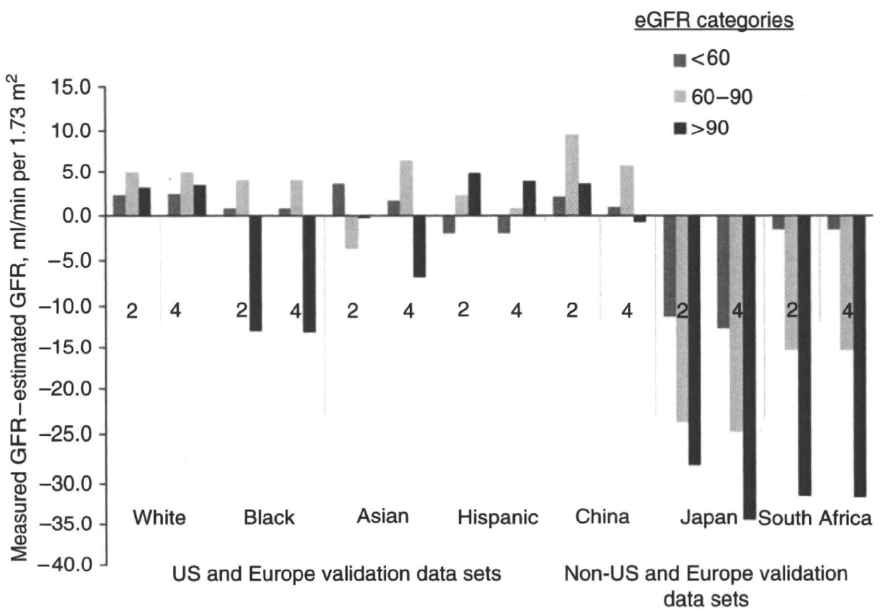

Figure 1 | Performance by level of estimated glomerular filtration rate (eGFR): Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) validation data set.

reflect variation in creatinine generation because of muscle mass or diet. The definition of the race coefficient as Black vs White and other in the MDRD Study does not account for differences in creatinine generation among other racial and ethnic groups. In the process of developing the CKD-EPI equation, we sought to develop an equation that better captures the variation in creatinine generation among racial and ethnic groups other than Blacks and Whites. The results of this process are described in this study. The four-level race equation that was developed is more accurate than the CKD-EPI (two-level race) equation in some, but not all, populations, and both equations demonstrated heterogeneous results within racial and ethnic groups across geographic regions. Given these results, we concluded that the four-level race equation was not sufficiently accurate to be implemented in clinical practice, and had selected the CKD-EPI equation with its two-level race variable.[10] Nevertheless, these results are informative for use of the two-level race CKD-EPI equation in these groups, and also suggest future research directions to derive generalizable racial and ethnic coefficients for GFR-estimating equations based on serum creatinine.

The coefficient for Blacks in the two- and four-level race term yielded a 15% higher eGFR for Blacks than for Whites at a given serum creatinine level, which is consistent with physiological data showing greater skeletal muscle mass in Blacks than otherwise equivalently matched White subjects.[14,15] Similarly, African Black athletes also have greater lean body mass compared with Whites.[16] Using either equation, the eGFR for Blacks in the CKD-EPI validation data set accurately estimated measured GFR. In contrast, these equations led to an overestimation of measured GFR by 12 ml/min per 1.73 m^2 in the South African data. This indicates a different relationship between serum creatinine and GFR for Black South Africans vs US and European Blacks, as shown previously for the MDRD Study equation

using these data[17] as well as in a separate population in Ghana.[18] This difference may be because of lower muscle mass in African Blacks compared with African Americans, potentially secondary to poorer diet or overall health, related to HIV infection or other chronic diseases. Indeed, the mean BMI in the Ghanaian and South African populations was lower than in the Blacks in the CKD-EPI validation data set. In a previous publication, we showed that the CKD-EPI equation overestimates measured GFR in people with low BMI.[5] Our data from South Africa, as well as the data from Ghana, demonstrated that GFR estimates are more accurate if the Black term is omitted. These data raise important questions about the appropriateness of use of the Black coefficient in the CKD-EPI and MDRD Study equations for GFR estimation in Blacks outside the United States and Europe.

The Asian coefficient in the four-level race equation translates into a 5% higher GFR at a given serum creatinine value compared with Whites and others. This is unexpected, given that some previous physiological and epidemiological data suggest that Asians have less muscle mass and lower dietary intake than Whites. For example, in an analysis of people in Pakistan, participants had lower mean creatinine excretion rates than those estimated for age- and gender-matched white individuals.[19] In other studies, Asians have been shown to have a higher percent body fat for the same level of BMI than Whites, suggesting lower levels of muscle mass.[20] The direction of the Asian coefficient is consistent with the modification of the MDRD Study equation for Chinese reported by Ma *et al.*,[8] whose data are included here as part of the non-US and Europe validation data set. Although the 5% higher eGFR was substantially lower than the 23% reported by Ma *et al.*,[8] they are both in contrast to the Japanese coefficient for the modification of the MDRD Study and CKD-EPI equations of 0.808 (ref. 9) and 0.8132 (ref. 21), respectively, which translate to a 19% lower GFR at a given serum creatinine.

The Asian coefficient in the four-level race equation led to more accurate GFR estimates in Asians in the CKD-EPI validation data set as well as in the Chinese data set, but neither the two-level or four-level equation resulted in accurate estimates in the Japanese. Both the Chinese and Japanese cohorts had a greater proportion of people with BMI $<20\,kg/m^2$ than the CKD-EPI development and validation data sets, but were similar to each other, suggesting that the overestimation of measured GFR in the Japanese cohort is not related solely to differences in levels of BMI. Factors other than muscle mass and diet may explain the difference between the Chinese and Japanese coefficients, such as differences in GFR measurement methods and the accuracy of creatinine calibration.[22] The countries of origin for the Asians in the CKD-EPI data sets are not known, and therefore we are not able to ascertain whether the Asian coefficient >1.0 in the four-level race CKD-EPI equation reflects Chinese origin. If future analyses establish that creatinine generation varies among Asian groups, then coefficients for subgroups of Asians in the CKD-EPI and other creatinine-based equations will need to reflect this variation.

The Native American and Hispanic coefficient resulted in a nonstatistically significant 1% higher eGFR for each serum creatinine value compared with Whites and others, and did not improve GFR estimation, suggesting that modification of the CKD-EPI equation may not be necessary for GFR estimation in Native Americans and Hispanics. To our knowledge, this is the only demonstration of the performance of the CKD-EPI equation in these groups. We are not aware of data on muscle mass in Native American and Hispanic populations. Data from NHANES (National Health and Nutrition Examination Survey) show a 5.3% lower mean level of serum creatinine for young healthy Mexican American men compared with Whites,[4] which has been interpreted as lower creatinine generation, but it may also reflect higher GFR. Furthermore, there is likely to be heterogeneity among Hispanic populations based on country of origin. There are only a small number of Native Americans and Hispanics in the CKD-EPI development data set and we do not have information on their country of origin.

The strengths of this study include the large diverse study population, with and without kidney diseases; calibration of the creatinine assays in each study to standardized values; rigorous and sophisticated statistical techniques for equation development; and evaluation of the equations in a separate data set of multiple studies that maximized external generalizability.

Our database had several limitations. First, it included only a small number of non-Blacks and non-Whites in both the CKD-EPI development and validation data sets. Nonetheless, the confidence intervals for the Asian and Native American and Hispanic coefficients were narrow, suggesting little variability among these groups in non-GFR determinants of serum creatinine. Second, the studies used a variety of methods to measure GFR that may have affected model evaluation. Finally, because we did not have information on country of origin for Asians and Hispanics in the CKD-EPI data sets, we grouped all Asians together and also grouped Hispanics and Native Americans, limiting a more nuanced analysis. Finally, the studies differed in their racial distributions, and hence the race effects cannot be entirely disentangled from study differences. Nevertheless, comparison of equations in a separate validation data set overcomes some of the limitations of differences among studies in patient characteristics and methods for measurement of GFR and serum creatinine, and provides support for the generalization of these results.

This study has several implications for clinical practice and research. First, the MDRD Study equation is currently widely used by clinicians, researchers, and public health officials, and is automatically reported by clinical laboratories whenever serum creatinine is ordered in the United States and Canada as well as in several countries in Europe.[23] In these countries, we suggest that the CKD-EPI (two-level race) equation could be used across a wide range of race and ethnicity, with the understanding that there is likely to be variation in accuracy of GFR estimates among and within

racial and ethnic groups based on factors associated with variation in creatinine generation, just as there is variation in accuracy within age and sex groups. Additional studies with a greater number and better characterization of participants from racial and ethnic minorities are necessary to develop more accurate estimates. Second, in geographic regions outside the United States and Europe, differences in creatinine generation within race and ethnic groups may limit the application of any creatinine-based estimating equation, unless the equation was specifically developed in that region. This limitation could possibly apply to immigrants of the same race and ethnicity from one region to another. Before recommending the CKD-EPI equation (or any creatinine-based estimating equation) in clinical practice, studies are required to determine whether modifications to the CKD-EPI (two-level race) equation are necessary.[8,21] Third, emphasis should be placed on investigation of filtration markers that may be less affected than creatinine by race and ethnicity, such as cystatin C and other novel markers.

In summary, racial differences in performance of creatinine-based estimating equation likely reflect geographic and ethnic differences rather than race *per se*. The four-level race equation was more accurate in some populations but not all. The CKD-EPI (two-level race) equation can be used in the United States and Europe across a wide range of race and ethnicity with appropriate attention to factors that affect creatinine generation.

MATERIALS AND METHODS

Sources of data and measurements

CKD-EPI is a research group funded by the NIDDK (National Institute of Diabetes, Digestive and Kidney Disease) to address challenges in the study and care of CKD, including development and validation of improved GFR-estimating equations by pooling data from research studies and clinical populations (hereafter referred to as 'studies').[10] The design and studies have been previously described and are briefly reviewed here.[10] We developed and internally validated the CKD-EPI equation in a database of 10 studies with a total of 8254 participants, divided randomly into separate data sets for development ($n = 5504$) and internal validation ($n = 2750$). The equations were then externally validated in a separate data set of 16 other studies with a total of 3896 participants. In the current report, we use the same data set as previously described for development and internal validation.[10] We also use the same external validation data set as previously described,[10] with the addition of data from Native Americans that were not available in the original report because of absence of creatinine calibration at the time of the original report, but now available to us (herein referred to as 'CKD-EPI validation data set') ($N = 4014$). We also evaluated the equations in three separate studies from outside of United States and Europe; two are from Asia[8,21] (referred to as 'China' and 'Japan') and one is from South Africa[17] (referred to as 'South Africa'), each of which has been previously described (herein referred together as 'non-US and Europe validation data sets').

GFR was measured using urinary clearance of iothalamate in the development data set and iothalamate and other filtration markers in the external validation data sets (Supplementary Appendix A and Appendix B). Serum creatinine values were calibrated to standardized creatinine measurements using the Roche enzymatic method (Roche-Hitachi P-Module instrument with Roche Creatininase Plus assay; Hoffmann–La Roche, Basel, Switzerland) at the Cleveland Clinic Research Laboratory (Cleveland, OH).[24,25]

Development and validation

Methods for development and validation have been previously described in detail.[10] In brief, we used least squares linear regression to relate measured GFR to serum creatinine and clinical characteristics available in the development data set. Predictor variables included serum creatinine, age, sex, and race in all equations. GFR was adjusted for body surface area.[26] GFR and serum creatinine were transformed to natural logarithms to reflect their inverse relationship and satisfy the assumption of a normal error distribution to stabilize variance across the range of GFR. We tested multiple forms of creatinine and age, and the final model includes a piecewise linear spline of log serum creatinine with a knot at 0.7 mg/dl in men and 0.9 mg/dl in women, and linear age.

Information on race and ethnicity was provided in the original study data. Race was defined as a two-level variable (Black vs White and other) and as a four-level variable (Black, Asian, Native American and Hispanic vs White and other). The specific origin of Asians was not specified in the original studies. The rationale for grouping Native Americans and Hispanics together is that the majority of non-Black Hispanics in the United States are from Mexico, and they are considered to be of mixed European-Native American descent.[27,28] The rationale for grouping others with White is that many of the other groups are defined as of Caucasian descent (for example, Arabs, non-Black, and non-Native American Hispanics). In some studies, information on ethnicity is not available, and it is possible that some Blacks or Whites were also Hispanics. We developed models in parallel using two- and four-level variables for race. Race groups were defined using a categorical variable with all levels necessarily included in the models using indicator variables. We selected models to bring forth from development into internal and then external validation based on analyses of the two-level race variable, with models using the four-level race variable brought along in parallel. For clarity of presentation, we will refer to the two equations as two- and four-level race equations.

Models created in the development database were first validated in the internal validation database. The development and internal validation data sets were then combined and equations were refit to yield more precise final coefficients to be used in subsequent analyses. Models were then evaluated in the CKD-EPI validation data set and a final two-level race model was selected using a prespecified series of steps, as has been previously described.[10] The four-level race variable model presented here is the parallel model to the final two-level race model, which is known as the CKD-EPI equation.[10] Results are also presented in the non-US and Europe validation data set by study.

Model performance

Performance of the equations was evaluated using similar metrics in both the development and two validation databases. Bias was expressed as the difference (mGFR–eGFR) and percent difference ($100 * [\text{mGFR} - \text{eGFR}]/\text{mGFR}$) between measured GFR (mGFR) and eGFR, with positive values indicating lower eGFR than mGFR (underestimation). Precision was expressed as interquartile range for the differences. Accuracy was expressed as P_{30} that takes into

account higher errors at higher values. We defined the probability of a large error as $1\text{–}P_{30}$.

Performance was evaluated within subgroups defined by the following clinical characteristics: age (<40, 40–65, and >65 years), sex, race (Black, Asian, Native American and Hispanic, and White and other), diabetes (yes, no), previous organ transplant (yes, no), and BMI (<20, 20–25, 26–30, and >30 kg/m^2). Level of eGFR was categorized as <60, 60–90, and >90 ml/min per 1.73 m^2.

Confidence intervals were calculated by bootstrap methods (2000 bootstraps) for difference, percent difference, and for P_{30}. Significance testing between metrics for each equation was computed using the Wilcoxon rank test on the bootstrapped estimates. Analyses were computed using R (Version 2, Free Software Foundation, Boston, MA) and SAS software (version, 9.1, Cary, NC). Smooth estimates of the mean in the figures were created using the lowess function in R.

The institutional review boards of all participating institutions approved the study.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Appendix A. Development and internal validation race/ethnic group, *N* (%).

Appendix B. External validation.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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Special Article Series: Vascular Pathology

Kawasaki Disease as a Systemic Vasculitis in Childhood

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Kawasaki disease is a disease of unknown etiology that most frequently affects infants and children under 5 years of age. Inflammation occurs in medium-sized muscular arteries throughout the body including the coronary artery, being classified as a systemic vasculitis syndrome. Histopathological investigations of Kawasaki disease have mainly focused on the coronary artery because it is directly associated with the cause of death. However, to identify the cause and pathology of Kawasaki disease, it is necessary to investigate lesions of whole organs. Thus, we attempted to review lesions in organs other than the heart and hypotheses of pathogenesis recently attracting attention.

Key Words: Kawasaki disease, pathology, systemic vasculitis syndrome, granulomatous angitis, infant

Introduction

Kawasaki disease was first reported as an acute febrile mucocutaneous lymph-node syndrome by Tomisaku Kawasaki in 1967.[1, 2] The prognosis of the disease was initially considered favorable, but the presence of fatal cases was clarified by a nationwide survey. On autopsy, systemic vasculitis was observed and a vasculitis-based aneurysm was formed in the coronary artery, clarifying that thrombotic occlusion of the lumen occurring in the aneurysm caused fatal ischemic heart disease.[3]

The diagnosis of Kawasaki disease is based on clinical signs and symptoms, which are classified as principal clinical and other clinical and laboratory findings.[4] The principal symptoms include: 1) fever persisting for 5 days or more; 2) bilateral conjunctival congestion; 3) changes in the lips and oral cavity: reddening of the lips, strawberry tongue, and diffuse infection of the oral and pharyngeal mucosa; 4) polymorphous exanthema; 5) changes in the peripheral extremities: initial stage = reddening of the palms and soles, and indurative edema, convalescent stage = membranous desquamation from the fingertips; and 6) acute nonpurulent cervical lymphadenopathy. At least five of the above six items must be satisfied for a diagnosis of Kawasaki disease. However, patients showing four of the principal symptoms can be diagnosed with Kawasaki disease when a coronary aneurysm or dilation is detected by two–dimensional echocardiography or coronary angiography.

The involvement of an infective factor in the development of Kawasaki disease has been assumed, but the cause is still unclear. A nationwide epidemiological survey of Kawasaki disease has been continued from 1970, and the total number of registered patients exceeds 240,000. The peak onset age is one year, and 70% of pediatric patients develop the disease at 3 years of age or younger. More than 10,000 patients have been reported annually in recent years, and the number of patients is still increasing.[5] After Furusho et al.[6] reported the efficacy of immunoglobulin, the incidence of coronary arterial complication decreased and the mortality was markedly reduced. However, 15% of pediatric patients do not sufficiently respond to immunoglobulin. The develop-

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Table 1 The incidence of arteritis in various organs throughout the body in Kawasaki diseasew

	Naoe[11]	Amano[12, 13]	Hamashima[14]	Landing[15]
Aorta	+	100%	82%	41%
Carotid a	+	75%		23%
Subclavian a	+	71%	67%	
Celiac a	+	79%	63%	
Iliac a	+	100%	93%	
Pulmonary a	59%	71%	50%	32%
Coronary a	95%	100%	95%	100%
Renal a	73%	80%	64%	55%
Mesenteric a	+	79%	86%	27%
Hepatic a	+	76%	44%	23%
Intercostal a	+	58%	60%	
Splenic a	11%	50%		50%
GI tract	10%			18%
Paratrachea	+			36%
Pancreas/ peripancreas	31%			36%
Adrenal / periadrenal	+			32%
Spermatic cord	+			41%
Testis	15%		67%	18%
Vagina	+			9%
Uterus	+			5%
Skeletal muscle				27%
Meninges	1%		36%	5%

ment of new therapeutic methods that provide alternatives to immunoglobulin is another task.[7–10]

The histopathological investigation of Kawasaki disease is still focused on the coronary artery, as in the past, because coronary arterial lesions are directly associated with fatality and their details have to be elucidated. Regarding organs other than the heart, many histopathological investigations were reported in the 1980s, but rarely reported thereafter as the number of autopsies of Kawasaki disease patients decreased. Moreover, many of these were abstracts of academic meetings or written in Japanese. To investigate the pathology and cause of Kawasaki disease, it is necessary to understand lesions of not only the heart but also the whole body. Thus, this report firstly aims at reviewing the histopathology of organs other than the heart, focusing on vascular lesions, in English. The histopathology of various organs we investigated is presented for your understanding.

Outline of Systemic Vascular Lesions

Whole body examination for Kawasaki disease as a systemic vasculitis was outlined by Naoe et al.,[11] Amano et al.,[12, 13] Hamashima et al.,[14] and Landing et al..[15] All reported that coronary arteritis occurred at the highest incidence but vasculitis developed at various sites throughout the body (**Table 1**). Naoe et al.[11] reported that vascular lesions of Kawasaki disease started in the tunica interna and externa of medium-sized muscular arteries, such as the coronary artery, whereas Amano et al.[12, 13] and Hamashima et al.[14] reported that vasculitis started in arterioles, venules and capillaries, and inflammation disseminated to large arteries including the coronary artery. The following points are consistent among Japanese researchers: Firstly, the histological characteristic of vasculitis in Kawasaki disease is proliferative granulomatous inflammation consisting of markedly accumulating monocytes/macrophages, but fibrinoid necrosis rarely occurs. Secondly, vasculitis in Kawasaki disease starts simultaneously with the onset, rapidly reaches an inflammatory peak, and then slowly remits and heals with cicatrization, showing a monophasic course. However, Landing et al.[15] observed late lesions corresponding to scars of vasculitis in about 1/3 of arteries in patients who died in the acute phase within 2 weeks after onset, and acute inflammatory findings (acute lesions) in about half of the arteries even at 3 months after the onset, showing that

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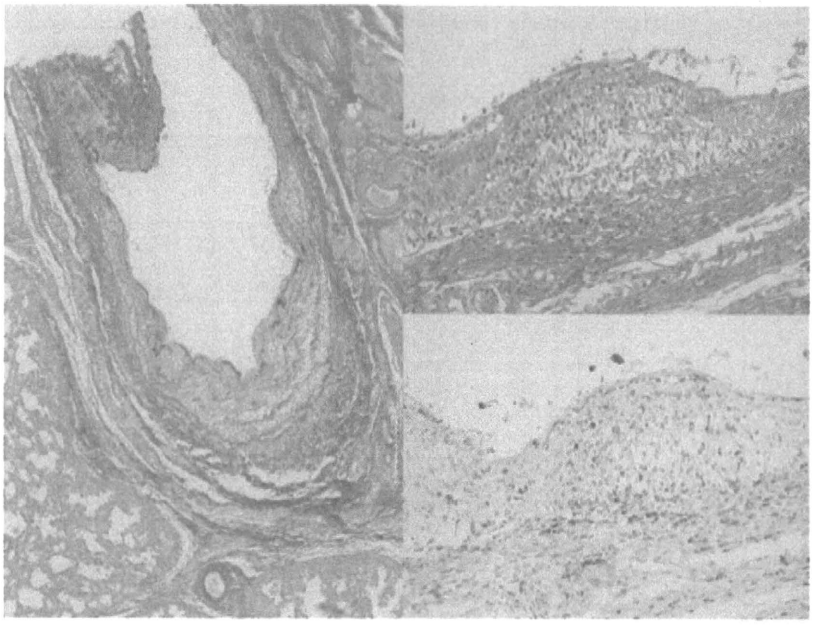

Fig. 1 Arteritis observed in the elastic type pulmonary artery. The tunica media was edematously thickened, and a small number of CD68-positive monocytes/macrophages infiltrated the vascular wall. Pulmonary arterial inflammation was milder than that of the coronary artery (**A**: elastic van Gieson stain, **B**: H&E, **C**: CD68).

vasculitis in the acute and cicatrical phases is mixed in Kawasaki disease. However, in our study, the course of Kawasaki disease vasculitis was synchronous throughout the body.[16] The mixed presence of acute- and cicatrical phase vasculitis is a histological feature of polyarteritis nodosa (PAN), suggesting that cases of PAN in childhood were included in the survey reported by Landing et al.

Lung

Shibuya et al. histopathologically investigated the pulmonary artery.[17] Panangiitis occurred in the pulmonary artery in 20 of 34 fatal cases (59%) within 60 days after onset, and all these were localized to the elastic pulmonary artery at sites up to the 4th branching. The earliest change in the pulmonary artery was edematous dissociation of the tunica media observed in a patient who died on the 13th illness day. The condition progressed to severe panarteritis on the 25–30th illness day (**Fig. 1**). After day 30, inflammation started to remit and scars were formed in patients who died at 3 months. No aneurysm or arterial dilatation was noted in the pulmonary artery, which may have been due to the low blood pressure.

In the acute phase of Kawasaki disease, interstitial lung shadows appear in some cases. On autopsy, interstitial changes were observed in 4 of 13 patients (31%) who died on the 29–57th illness day. Histologically, the changes corresponded to diffuse alveolar damage (DAD) (**Fig. 2**), but there was no apparent correlation between pulmonary arteritis and the interstitial changes.[18]

Kidney

The incidence of panarteritis in the kidney varies among reports.[19–21] Asaji et al. [19] observed panangiitis or its scars in arteries in the kidney on autopsy in 35 of 48 Kawasaki disease patients (73%) who died 6 days-11 years after the onset. Arteritis developed as edematous dissociation of the tunica media in a patient who died on the 13th illness day, and granulomatous and proliferative inflammation were noted in patients who died on days 17–28 (**Fig. 3**). Inflammation remitted after day 30. Compared to coronary arteritis, arteritis in the kidney developed several days later and inflammation was milder. Panangiitis was localized in the interlobar arteries and rarely occurred in the arcuate and interlobular arteries. The occurrence of renal aneurysm is recognized, and the development of renal hypertension due to renal arterial stenosis has been reported,[22] but descriptions concerning the renal artery in autopsy cases are lacking.

Regarding glomerular changes, the presence of segmental or global glomerulosclerosis was described in many reports.[19–21] However, these are considered to be physiological changes occurring with childhood development, called infantile glomerulosclerosis, not specific to

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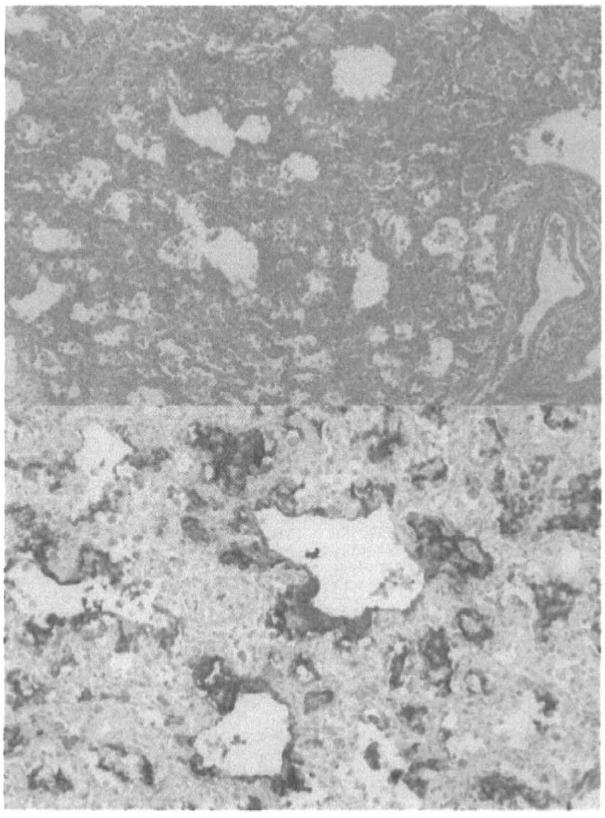
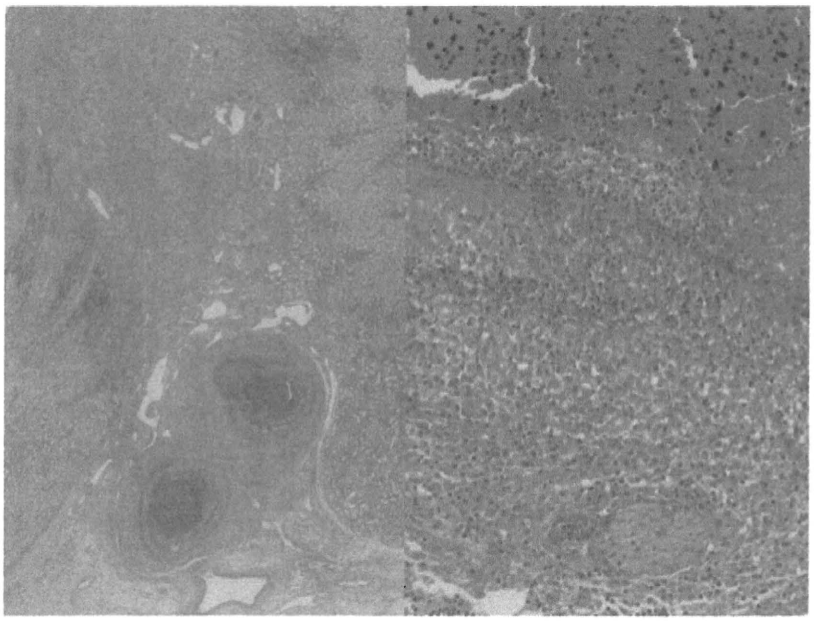


A

B

Fig. 2 A: Interstitial pneumonia observed in a patient who died 29 days after the onset of Kawasaki disease. Hyaline membranes appeared over a wide area (H&E).
B: The hyaline membranes positively reacted with anti-surfactant apoprotein antibody.



A | B

Fig. 3 Proliferative and granulomatous arteritis of the interlobar artery in the renal hilar region. The vascular lumen was obstructed with a thrombus. (H&E, A: × 2, B: × 20)

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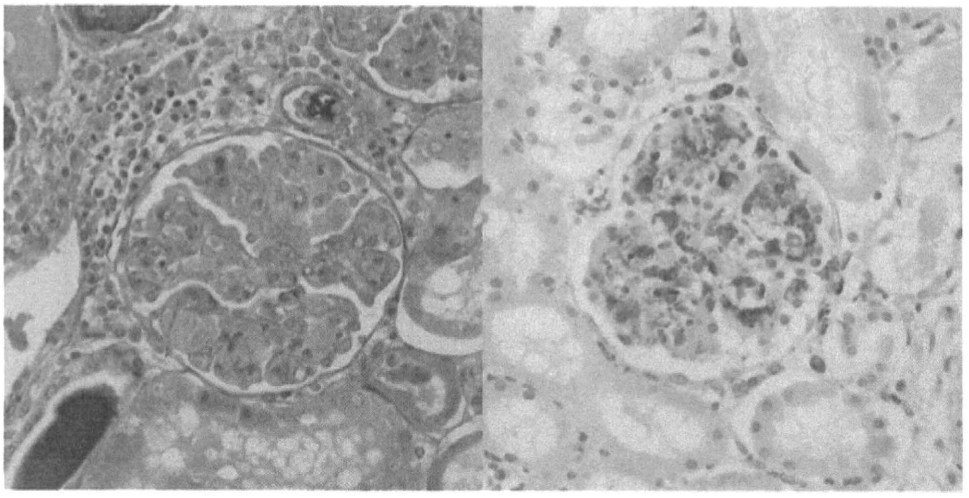


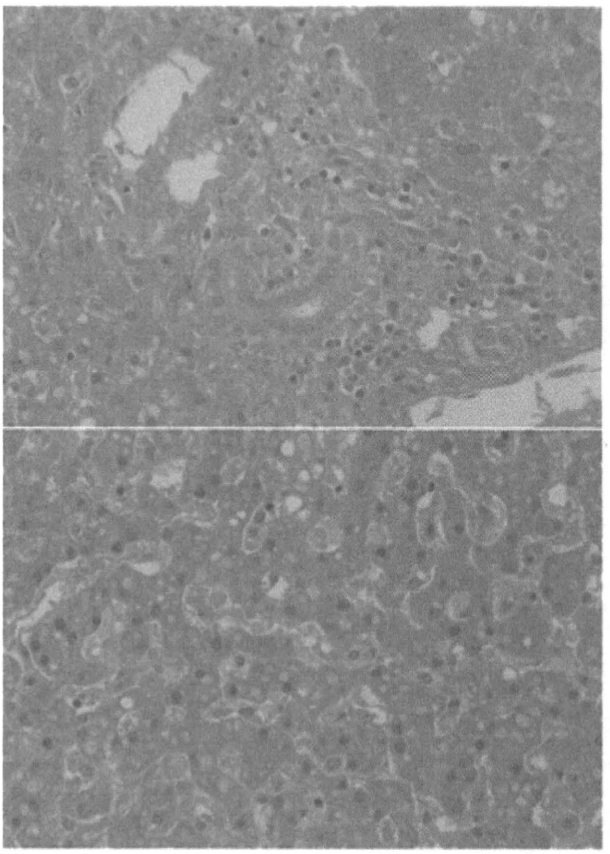
A | B

Fig. 4 The renal glomerular lesion in a sole case in which we observed an intracapillary proliferative change. Many CD68-positive monocytes/macrophages were present in capillary blood vessels. Generally, no renal glomerular lesion develops in Kawasaki disease (A: PAM stain, B: CD68).

Kawasaki disease. Focal segmental mesangial proliferation was also reported as another glomerular change noted in Kawasaki disease.[20, 23] We observed many monocytes/macrophages in the glomerular capillary, showing a feature of intracapillary proliferative glomerulonephritis, in only one case (**Fig. 4**), but no immune complex deposition in glomeruli was noted. The observation of tubular changes in 8% of cases was reported.[19]

Liver

Liver dysfunction occurs at a high incidence in the acute phase of Kawasaki disease. Tanaka et al.[24] performed liver biopsy in 19 patients at 7–36 days after onset, and observed the fatty and edematous degeneration of hepatocytes and severe inflammatory cell infiltration in the portal area in most patients (**Fig. 5**). Portal area vascular inflammation was unclear, and hepatic changes were assumed to be toxic rather than circulatory impairment. Ohshio et al.[25] also reported that inflammatory cell infiltration in the portal area was observed in the acute phase at a high frequency, and cholangitis and pericholangitis were more noticeable than vasculitis of the portal area.



A
B

Fig. 5 A: Mild lymphocyte and plasma cell infiltration was noted in the portal area of the liver.
B: Droplet fatty degeneration in hepatocytes.

Gallbladder

Suddleson et al.[26] observed swelling of the gallbladder in 16 of 117 patients (14%) with acute Kawasaki disease

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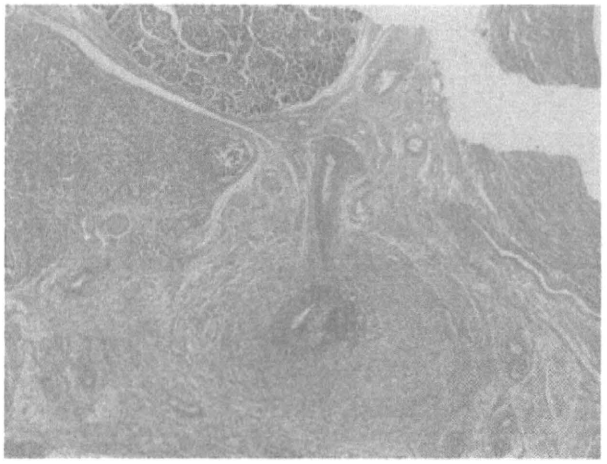

Fig. 6 Inflammation of the medium-sized muscular artery in the pancreatic interstitium.

Fig. 7 Multiple ulcers in the small intestine

on ultrasonographic examination. The gallbladder inflammation remitted as Kawasaki disease resolved, showing that surgical excision of the swollen gallbladder in Kawasaki disease is unnecessary. Masuda et al.[27] histopathologically investigated gallbladders that were surgically excised based on a diagnosis of cholecystitis in 4 patients with acute Kawasaki disease, and observed characteristic non-specific acalculous cholecystitis. Regarding vascular changes, perivascular cell infiltration was observed, but panangiitis was noted only in one of the 4 cases, and the panangiitis occurred in a artery with a 400-μm diameter in the subserosal layer.

Spleen and Pancreas

Pancreatic vascular lesions developed in 14 of 45 patients (31%), and the lesions were located at sites up to the pancreatic interlobular arteries. Vasculitis started on the 10th illness day, reached an inflammatory peak at about day 28 (**Fig. 6**), and then healed while fibrous intimal thickening remained. In the spleen, arteritis was noted in arteries in the hilar and trabecular regions, and the histological findings were similar to those in the pancreas.[28] Yoshioka et al.[29] reported that inflammatory cell infiltration in the pancreatic duct and its surrounding and vasculitis were characteristic, and the inflammatory findings of the pancreatic duct were marked in acute cases by day 27.

Gastrointestinal Tract

Kurashige et al.[30] histopathologically investigated gastrointestinal lesions in 31 autopsy cases of Kawasaki disease. Vasculitis was noted in 3 patients, but all were localized in arteries in the subserosa, not between mucosal and muscular layer, showing a difference from PAN with regard to the distribution of lesions. On the other hand, ulcers graded as UI-III were present in 3 cases (**Fig. 7**). In addition, the reactive hyperplasia of lymphoid follicles was often noted in mucosa at the end of the ileum.

Lymph Node

Cervical lymph node swelling is one of the principal clinical findings described in the diagnostic guidelines for Kawasaki disease, and it is present in 70% of acute cases. Histopathologically, lymph node swelling in Kawasaki disease was a nonspecific reactive change in many cases but characterized by focal necrosis which started from the marginal sinus.[3, 31–33] Necrotized regions were mixed with karyorrhexis, and enlarged endothelial cells, luminal obstruction and fibrin thrombi were noted in micro blood vessels continuous with the necrotized lesions (**Fig. 8**). Some researchers consider that the cause of necrosis is microinfarction, while others consider changes with inflammation.[32, 33] We suggest that necrosis is due to infarction, and extracapsular dissemination of inflammation is the point of differentiation from histiocytic necrotizing lymphadenitis (Kikuchi-Fujimoto's disease).

Skin

Changes in the skin are also a principal clinical find-

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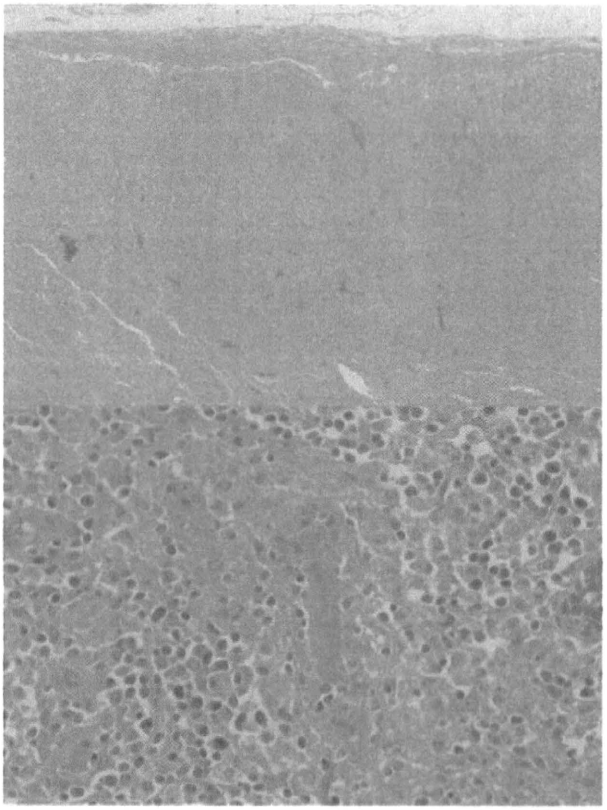

Fig. 8 A: Small necrosis directly below the lymph node capsule
B: Fibrin thrombus in a small blood vessel

ing, as well as lymph node swelling, described in the diagnostic guidelines, and many pathological investigations have been reported. Many points are consistent among these reports, summarized as follows: Skin lesions are characterized by markedly inflammatory edema accompanied by vasodilatation in the dermal papillary layer and fibrin exudation.[34–36] These changes are marked in scars of BCG vaccination, and granulomatous inflammation was noted in some cases.[37] Endothelial cells enlarged and were surrounded by infiltrating monocytes/macrophages and CD4-positive T cells, but very few neutrophils and B cells were present. No panangiitis was noted. It has been reported that IL-1α and TNF-α were strongly positive in the acute phase but negative in the recovery phase on immunohistological investigation.[38]

Other Organs

In the intercostal arteries which directly branch from the aorta, similarly to the coronary artery, lesions develop at the proximal site of the arteries.[39] The histology of vasculitis is also similar to that of the coronary artery, and aneurysms may be formed.

Regarding conjunctival changes, one of the main signs of Kawasaki disease, Burns et al. [40] reported that these were nonspecific, such as vasodilatation and mild infiltration by lymphocytes and plasma cells.

In the salivary glands, severe inflammatory cell infiltration by lymphocytes, plasma cells, and neutrophils occurs around the acinus and excretory ducts in the acute phase and remit in the recovery phase.[41]

Testicular vascular lesions and capsulitis were observed in cases in the acute phase, but no interstitial or parenchymal inflammation was noted in the testis.[42]

In nerve tissue, aseptic chorio and/or leptomeningitis was noted in about half of autopsy cases of Kawasaki disease, and mild or moderate inflammatory cell infiltration by lymphocytes, monocytes/macrophages, and a few neutrophils was observed. Edema in perivascular or perineuronal areas and a localized spongy state were occasionally noted. Regarding cerebral blood vessels, perivascular inflammatory cell infiltration was observed, but panangiitis was not.[43]

Pathogenic Hypothesis Recently Attracting Attention

Rowley et al. observed that IgA plasma cells infiltrated vasculitis lesions with many monocytes/macrophages and CD8 T lymphocytes in autopsy cases of Kawasaki disease.[44] These IgA plasma cells also infiltrated non-vascular tissues throughout the body, such as the bronchus, pancreas, and renal tissue.[45] They hypothesized that a pathogen, probably a virus, which invades via the respiratory or digestive organs is processed by the lymph apparatus in the organ, local B lymphocytes differentiate into precursors of IgA plasma cells, and then IgA-producing plasma cells reach not only the coronary artery and heart muscle but also various organs throughout the body. IgA plasma cells are oligoclonal or antigen-driven.[46] Synthetic antibodies were produced in vitro by cloning α and κ variable-region genes prevalent in the Kawasaki disease arterial wall into immunoglobulin expression vectors and producing the antibodies in tissue culture. Employing this antibody, autopsy preparations from Kawasaki disease patients were immunohistochemically investigated. In addition to monocytes/macrophages in vascular lesions, a substance reactive with

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the antibody was present in the cytoplasm of bronchial ciliated epithelial cells.[47] The cytoplasmic inclusion bodies in bronchial epithelium could be identified by H&E staining and observed as an electron-dense non-structured spheroid substance under an electron microscope.[48] Analysis of the structure of this cytoplasmic inclusion body is underway.

Nagata et al.[49] immunohistochemically investigated biopsy specimens of small intestinal mucosa, and assumed that antigen which strongly activates CD4-positive cells in intestinal mucosa and intestinal epithelial cells is associated with the development of Kawasaki disease. To investigate this hypothesis, they analyzed bacteria isolated from the oral cavity and duodenal mucosa, and clarified that gram-positive cocci with superantigen activity and gram-negative bacteria, which produce heat shock protein 60 and induce inflammatory cytokine production in peripheral blood monocytes in pediatric patients, were isolated from children with Kawasaki disease at a high rate, suggesting that these microorganisms act together and induce Kawasaki disease.[50]

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