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第9回鳥取大学小児神経学入門講座・第30回米子セミナーのお知らせ

会　　期　平成23年9月23日（金）～24日（土）
会　　場　鳥取大学医学部臨床講義棟・ほか
小児神経学入門講座
①小児の神経変性疾患の鑑別
大野耕策（鳥取大学医学部脳神経小児科）
②小児てんかんの治療
平岩里佳（東部島根医療福祉センター）
③脳波の読み方
前垣義弘（鳥取大学医学部脳神経小児科）
④学習障害，とくに発達性読み書き障害の診療
小枝達也（鳥取大学地域学部）
⑤よくみる小児神経疾患の画像
金崎佳子（鳥取大学医学部放射線科）
⑥小児のリハビリテーション
北原　佶（鳥取県立総合療育センター）
⑦グループレクチャー
⑧教育講演「小児の認知機能に関する生理学的研究：刺激や解析法の工夫を通じて歩んできたこと」
稲垣真澄（国立精神・神経医療研究センター精神保健研究所知的障害研究部）
米子セミナー　①症例検討会（演題募集）
②画像検討会（演題募集）
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冠疾患誌 2011; 17: 66–74

冠動脈障害を有する川崎病既往者の冠循環動態および侵襲的治療前後の冠循環動態を考察する

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Ogawa S, Ochi M: Consideration of coronary hemodynamics in Kawasaki disease patients with coronary artery lesions and estimation of hemodynamic change before and after surgical treatment. J Jpn Coron Assoc 2011; 17: 66–74

I. はじめに

川崎病の主たる病態は，全身の血管炎であり，最近の初期治療法の改善により冠動脈障害は減少傾向にあるが，未だに，冠動脈拡大が2.3%，冠動脈瘤が1.0%，巨大冠動脈瘤が0.35%，さらに冠動脈狭窄が0.06%に認められている[1]．川崎病の原因は不明であるが，本邦では病歴から川崎病であったと考えられる最も早期の症例は1950年と報告されており[2]，戦後の病気と考えられる．戦後日本にもたらされた食・住環境の変化，大気汚染などの環境の変化，予防接種などが間接的要因として候補に上がる．なお，現在成人に達した川崎病既往者はすでに10万人を超え，その多くが急性期治療としてγグロブリン大量療法を受けていない．その当時の冠動脈障害発生率である20%という数字を当てはめてみると，すでに約20,000人が冠動脈後遺症を抱えて成人期を過ごしていることになる．

川崎病の冠動脈障害は冠動脈瘤を主体とする拡張性病変，瘤前後または瘤間に存在する狭窄性病変，および瘤内の血栓閉塞に伴う閉塞性病変，そこに側副血行路が関与し，複雑な冠血行動態を呈する．さらに，成人期に達すると生活習慣病が加わり，粥状動脈硬化症の合併が危惧される．

本稿では冠動脈障害を有する川崎病既往患者の冠循環動態を明らかにし，現在外科的治療として行われているCABG術前後および当院にて行っている冠動脈瘤縫縮術前後における冠血行動態につき概説する．

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II. 川崎病における冠動脈障害の特徴

1. 川崎病の急性期から回復期に認められる冠動脈障害の特徴

冠動脈瘤を主体とする拡張性病変である．現在，免疫グロブリン大量療法を中心とする初期治療の効果により冠動脈瘤の発生頻度は減少した．急性期において特に問題となるのはいわゆる巨大冠動脈瘤(巨大冠動脈瘤の定義：5歳未満では内径8 mm超，5歳以上では近傍の健常内径の4倍以上)の合併である．やや発生頻度は減少傾向にあるが，現在でも瘤内血栓塞栓による急性心筋梗塞や，瘤の急激な増大に伴う破裂などにより致命的となる場合もある．

2. 回復期以降，発症より数年内の経過

冠動脈瘤の入口部，出口部，複数の冠動脈瘤があればその瘤間，さらに稀には瘤内に狭窄性病変が出現する場合がある．血管炎の後には血管炎修復のためのpositive remodelingが惹起される．病理組織学的には中膜の血管平滑筋の内膜への迷入および増殖，さらに壁在血栓の基質化などが相まって内膜の肥厚がもたらされる．一方，内膜の急激な過増殖が起こることにより狭窄性病変が出現し，狭窄の程度によっては有意な心筋虚血病変が惹起される．また，瘤内の血栓による完全閉塞や側副血行路の形成，さらに，微小血栓による閉塞や血管炎に伴う微小血管内皮細胞傷害などによると考えられる微小冠循環障害[3,4]などさまざまの病変を抱える．

3. 発症より数年後以降の遠隔期

発症数年後には冠動脈病変部位を中心に更なる血管再構築が起こり，石灰化も加わり，障害部位はsclerotic な病変に変化していく．このような冠動脈障害を合併する患者では，成人期になり肥満，高血圧，糖尿病，脂質代謝異常，高尿酸血症，喫煙などの動脈硬化の発症・進展の危険因子を有することにより粥状動脈硬化を合併し，acute coronary syndromeを発症することが危惧される．

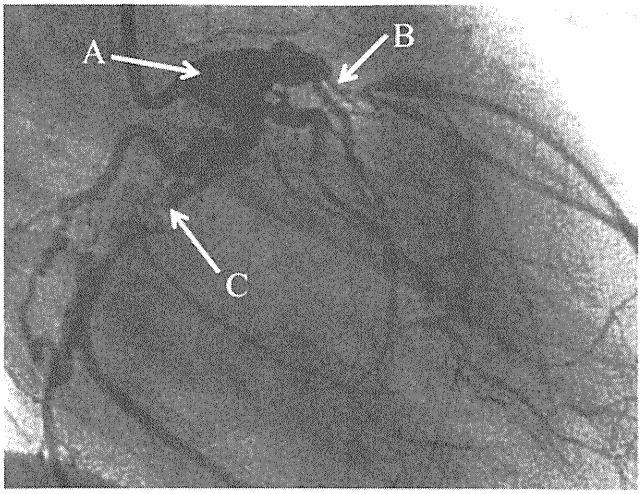

図1　川崎病後の冠動脈後遺症
A：冠動脈瘤，B：狭窄性病変，C：完全閉塞後の再疎通

以上，川崎病の冠動脈病変は急性期から遠隔期にかけて多彩である．川崎病の冠動脈病変は1枝に限らず複数枝にわたり，また1枝に孤立する病変ばかりでなく，複数カ所存在することも多く，非常に複雑な冠血行動態を呈する．このような複雑な冠循環動態の把握は通常の画像診断だけでは不十分であり，レオロジーの面からの機能的評価が重要となる(図1)．

III. 冠動脈障害を有する症例の冠循環動態の機能的評価

高感度圧センサー（RadiMedical社製のPressure Wire™）または，超音波探触子を装着したガイドワイヤー(FloWire XT, Cardiometrics Inc.)や1本のガイドワイヤーに圧およびドップラの両方のセンサーが装着されたガイドワイヤー(ComboWire, VOLCANO Corporation, USA)を用いて，冠血行動態を評価することは診断ばかりでなく，治療法の決定や治療効果の判定にも極めて有用である．これらの方法を用いて冠血行動態を評価する際に有用と思われる指標を表1に列挙した(表1)．

1. ドップラワイヤーを用いた冠血行動態の評価

小児における健常冠動脈の血流波形，APV(time-averaged peak flow velocity)，CFR(coronary flow reserve)の基準値を示す(表2)．われわれの検討では枝別および年齢の違いによる諸値に有意差は認められず，小児におけるAPVの基準値を15 cm/secに，CFRの基準値を2.0に設定した[5]．一方，成人領域におけるCFRの正常値の報告は概ね2.0以上であり[6-8]，小児における値は成人領域の値と同じであり，小児期から成人期にかけて年齢による差異はないことが確認された．

さらに，APVおよび血管径から以下の近似式にてshear stressを算出することが可能である．剪断応力(shear stress)は主として血管内皮細胞に作用し，血管内皮作動性物質を介して血行動態に多大な影響を及ぼす血流に起因するメカニカルストレスである．

Shear stress ≒ (4×μ×APV) / R

なお，μ(血液粘度)=3 cp (cp=0.003 Pa･sec, 1 Pa=10 dyne/cm^2)[9]，R：血管の半径

冠動脈枝別に検討したが有意差はなく，40 dye/cm^{2}をこの方法における小児の基準値とした(表2)．なお，われわれが測定できるAPVは血流の中央部での値であり，管壁では中央部よりも低下している．従って血管壁に近い部位のshear stressはより低い値となる．

2. プレッシャーワイヤーを用いた冠血行動態の評価

プレッシャーワイヤーにより評価可能な冠血行動態指標はFFRmyo(fractional flow reserve：心筋部分血流予備量比)である．血管拡張薬により末梢血管が最大に拡張した状態では血管抵抗は最小となり，冠血流と冠内圧は直線的な関係となる．従って，末梢冠動脈最大拡張時の冠動脈平均内圧(Pd)，冠動脈入口部平均圧(Pa)の圧比はその間に存在する病変(主として狭窄性病変)により減少した血液量を表す．つまり，FFRmyoの値の有意な低下は，その当該冠動脈の灌流心筋領域の虚血を示唆する．

健常と思われる各冠動脈枝に挿入し，FFRmyoを算出した[5](表3)．冠動脈枝別および年齢の違いよる差異は認められず，0.75未満を小児における異常値に設定した．成人領域におけるFFRmyoの異常値の報告は，0.75未満であり[10]，小児においても成人領域と同様の基準値が得られた．成人領域においては，FFRmyo値の0.75はIVUSからみた最小の血管内腔断面積3.0 mm^2，および面積狭窄率0.6と良好な相関関係が認められるとの報告があり[11]，

表1

用いる方法	検討した指標
Flow wire	血流波型，APV，CFR，shear stress
Pressure wire	血管内圧，FFRmyo
Flow and pressure wire (Combo Wire)	血管抵抗，その他Pressure wireおよびFlow wireとしてのそれぞれの指標(APV，CFR，FFRmyo，shear stress)

APV: time-averaged peak flow velocity(時間平均血流速度)，CFR: coronary flow reserve(冠血流予備能)，FFRmyo: myocardial fractional flow reserve(心筋部分血流予備量比)

表2

冠動脈枝別の血流パターン，APV，CFR

冠動脈枝	症例数	発症時月齢	血流パターン	APV (cm/sec)		CFR
				安静時	負荷時	
左前下行枝群	132	11.3±3.2	100% pulsatile	23.11±3.8	59.2±5.7	2.59±0.28
回旋枝群	110	11.6±3.1	100% pulsatile	23.8±4.0	60.2±5.9	2.58±0.32
右冠動脈群	130	11.2±2.6	100% pulsatile	24.0±3.7	60.5±6.4	2.63±0.30

冠動脈枝別の shear stress

冠動脈枝	症例数	APV (cm/sec)	血管径	Shear stress (dyne/cm^2)
左前下行枝群	54	24.1±4.2	2.9±0.6	56.3±6.8
回旋枝群	43	25.1±5.2	2.8±0.4	54.8±6.3
右冠動脈群	61	25.5±4.3	3.0±0.7	61.4±7.8

表3

冠動脈枝別の FFRmyo

	症例数	発症時月齢	冠動脈入口部平均圧(負荷時)	冠動脈平均内圧(負荷時)	FFRmyo
左前下行枝	112	12.4±3.4	61.4±4.4	55.6±6.1	0.93±0.08
回旋枝	94	13.3±4.1	62.1±3.7	56.1±5.1	0.90±0.06
右冠動脈	131	12.8±4.6	64.3±5.1	58.5±4.2	0.91±0.05

冠動脈枝別の末梢血管抵抗

冠動脈枝	症例数	冠内圧 (安静時) (mmHg)	APV (安静時) (cm/sec)	血管抵 (安静時)	抗冠内圧 (負荷時) (cm/sec)	APV (負荷時) (mmHg)	血管抵抗 (負荷時)
左前下行枝群	35	67.8±7.2	23.3±4.0	2.91±0.52	55.6±6.1	56.7±5.6	0.98±0.23
回旋枝群	28	65.9±5.8	23.7±3.9	2.78±0.48	56.1±5.1	58.8±5.5	0.95±0.34
右冠動脈群	39	69.3±7.8	24.2±3.8	2.88±0.43	58.5±4.2	59.1±6.8	1.00±0.36

形態学的な評価と機能的な評価の一致が認められると同時に，FFRmyo<0.75 を異常とする妥当性が得られている．

3. ドップラワイヤーおよびプレッシャーワイヤーより得られる末梢血管抵抗

冠動脈血流量・冠動脈内圧比より末梢血管抵抗を算出することが可能である．われわれは冠血流量とよく相関する APV を用いて APV・平均冠動脈内圧比を算出し一種の末梢血管抵抗として臨床に供している．安静時および血管拡張時の末梢血管抵抗を算出した．安静時は 4.0，血管拡張時は 2.0 をこの方法による基準値として設定し，これら以上を末梢血管抵抗の異常値とした(表3)．

4. 冠動脈障害の違いによる冠血行動態の変動

(1)有意な狭窄性病変を合併していない冠動脈瘤内およびその遠位部における血行動態

① 瘤内における血行動態

瘤内(拡張性病変内)の血行動態を見てみると，小冠動脈瘤(5歳未満では内径 4 mm 未満，5歳以上では近傍の健常内径の 1.5 倍未満)内の血流波形パターンは全例拍動性であり，APV，CFR，shear stress も正常範囲内であった．一方，中冠動脈瘤(5歳未満では内径 4 mm 以上 8 mm 以下，5歳以上では近傍の健常内径の 1.5 倍以上 4 倍未満)内では主として瘤の内径の増大により血流波形パターンは拍動性パターンから乱流パターンに変化した．また，APV，CFR，shear stress，末梢血管抵抗共に瘤の内径の増大に伴い一部異常値を呈した．一方，巨大冠動脈瘤ではほぼ全例が乱流パターンを呈し，APV は 10 cm/sec 以下，CFR は 1.5 以下，shear stress も 10 dyne/cm^2 以下と有意に低下した[12]．また，末梢血管抵抗もほとんどの症例で異常値を呈した．しかし，FFRmyo においては有意な変動は認められなかった(表4)．

以上の結果は冠動脈瘤内，特に巨大瘤内では灌流圧の低下は認められないが shear stress が極めて低下し，著

表4

瘤内における血流パターン，APV，CFR，shear stress，FFRmyoおよび末梢血管抵抗

	n	血流パターン	APV (cm/sec)	CFR	Shear stress (dyne/cm^2)	FFRmyo	血管抵抗	
							安静時	負荷時
小冠動脈瘤群	44	100% pulsatile	23.1±3.8	2.20±0.20	42.2±5.1	0.90±0.07	2.4±0.2	1.4±0.2
中冠動脈瘤群	36	77.8% pulsatile 22.2% turbulent	18.5±4.6	1.78±0.48	38.6±7.8	0.86±0.06	2.5±0.7	1.7±0.3
巨大冠動脈瘤群	21	4.8% pulsatile 95.2% turbulent	8.6±2.8*	1.10±0.28*	4.2±2.8*	0.83±0.08	5.8±0.6*	5.3±0.7*

*$p<0.05$ vs. 他冠動脈瘤群

瘤末梢における血流パターン，APV，CFR，Shear stress，FFRmyoおよび末梢血管抵抗

	n	血流パターン	APV (cm/sec)	CFR	Shear stress (dyne/cm^2)	FFRmyo	血管抵抗	
							安静時	負荷時
小冠動脈瘤群	43	100% pulsatile	23.5±3.2	2.25±0.19	48.3±4.9	0.89±0.07	2.3±0.3	1.2±0.4
中冠動脈瘤群	34	77.8% pulsatile 22.2% turbulent	19.2±3.8	1.82±0.46	43.8±5.9	0.88±0.08	2.5±0.7	1.5±0.3
巨大冠動脈瘤群	18	4.8% pulsatile 95.2% turbulent	10.2±3.2*	1.18±0.33*	12.1±3.2*	0.84±0.09	4.9±0.9*	4.4±0.7*

*$p<0.05$ vs. 他冠動脈瘤群

しい血管内皮細胞機能障害が惹起されていることが伺える．血管炎に伴う内皮細胞傷害に加えて，血行動態の異常に伴う内皮細胞傷害も相まって，巨大瘤内では重篤な血管内皮細胞傷害が起こっていることが危惧される．血管内皮細胞の機能低下は，血管収縮性を亢進させ，抗血栓作用，抗炎症作用，抗線維化作用，抗酸化作用，抗動脈硬化作用などを減弱させる．特に川崎病後の冠動脈瘤内では血栓形成が一番の問題となる．Shear stressの低下は血管内皮細胞を通して，プロスタグランジンI_2やNOの産生障害[13, 14]を惹起し，血小板凝集能を亢進させる．また，組織因子(TF)活性[15]やhuman protease-activated receptor-1の発現の増加を促し[16]，トロンボモジュリンの発現を抑制することなどにより凝固能を亢進させる[17]．さらに，shear stressの低下に伴い，組織プラスミノーゲンアクチベータ(t-PA)の産生障害[16, 18]や，プラスミノーゲンアクチベータインヒビター-1(PAI-1)の産生亢進により線溶系は抑制される．血小板凝集能および凝固能の亢進，線溶系の抑制などにより容易に血栓が形成される．従って，冠動脈瘤内，特に巨大瘤内のshear stressを中心とする血行動態を十分に把握することはその後の治療戦略を立てる上で重要となる．ただし，瘤内径が8 mmを超えるような瘤でもその形状により，血流波形パターン，APV，CFR共に正常である場合もある．従って，単に形態的に巨大瘤といっても血行動態的にはほぼ正常である場合も存在し，それらを層別化する上でも，これらの機能的評価は有用である．

② 瘤の末梢における血行動態

血流波形は冠動脈瘤内と同様のパターンを呈した．APV，CFRの値も瘤内とほぼ同様であったが，shear stressは冠動脈の内径が拡大した瘤内に比し有意に小さいため瘤内の値よりも高値を呈した．

一方，FFRmyoを検討してみると，瘤の大きさ，形状の如何にかかわらず有意な狭窄性病変がない限り正常範囲内であった．さらに，巨大瘤を有する冠動脈枝の末梢血管抵抗もばらつきはあるものの有意に高値を呈した．つまり，巨大冠動脈瘤の末梢部位では有意な狭窄性病変がなくとも灌流血液量の低下に伴う血管内皮機能障害，心筋虚血，さらに微小冠循環障害の存在が示唆された(表4)．

③ 狭窄性病変の遠位部における血行動態

負荷心筋シンチ所見より心筋虚血を伴う冠動脈狭窄群と心筋虚血を伴わない狭窄群に分類し，CFR，FFRmyo，shear stress，末梢血管抵抗の各指標につき比較検討した．虚血群は全例90%以上の狭窄度を有していた．一方，非虚血群の中には当該冠動脈が90%以上の狭窄性病変を有している症例が約10%含まれていたが，これらの症例では十分な側副血行路の発達が認められている．小児では有意な狭窄性病変に伴い，早期に側副血行路が出現する場合が多く，このような症例の狭窄病変の評価は形態学的評価だけでは十分ではない．表5に示すように，虚血群においては，CFR，FFRmyo，shear stress，末梢血管抵抗ともに，非虚血群に比し有意に変動し，かつ，その多くが設定した基準値を逸脱していた(表5)．以

表5　狭窄性病変の末梢部における APV，CFR，shear stress，FFRmyo および末梢血管抵抗

	症例数	APV	CFR	Shear stress	FFRmyo	血管抵抗(負荷後)
虚血群	31	13.2±3.4*	1.21±0.33*	14.3±2.8*	0.61±0.07*	3.8±0.5*
非虚血群	43	48.7±4.8	2.21±0.16	39.7±5.4	0.84±0.06	1.3±0.4

*p<0.05 vs. 非虚血群

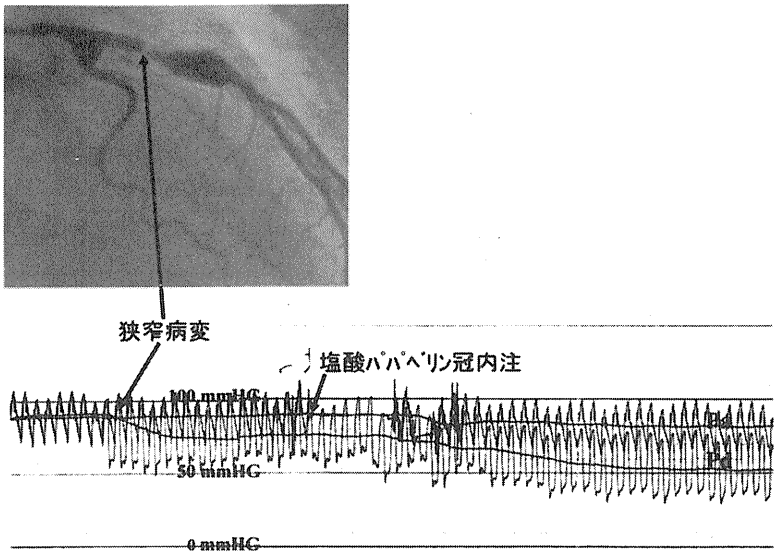

図2　狭窄性病変における FFRmyo を用いた重症度の評価
左前下行枝の瘤間に約 90%の局所性狭窄が認められる．
ガイディングカテーテルによる冠動脈入口部圧，プレッシャーワイヤーによる冠動脈内圧を同時記録．プレッシャーワイヤーが狭窄部位を通過後有意に冠動脈内圧は低下．塩酸パパベリンの冠動脈内注により更に低下し，FFRmyo は 0.65 と異常値を呈した．
FFRmyo: fractional flow reserve

上より，側副血行路を含む狭窄性病変の血行動態の評価には，ドップラワイヤー，プレッシャーワイヤーから得られる諸指標の検討が有用と思われる．これらの指標が異常値を呈する狭窄性病変末梢部位の灌流血液量は減少し，内皮機能障害，心筋虚血が惹起されていることが推察される．さらに灌流圧も低下しているが，それを上回る灌流血液量の低下が起こり，末梢の血管抵抗は上昇すると考える．狭窄性病変を評価する上において用いる指標としては FFRmyo の方が，CFR に比し，心拍数や心収縮能，灌流圧などによる影響を受けにくく優れている(図 2)．

IV. 川崎病既往者の成人期における問題点

慢性持続性血管炎は動脈硬化発症の危険因子の一つある．従って全身性血管炎である川崎病既往者が成人期を迎えた際に，健常人に比して有意に動脈硬化を合併しやすいかどうかが問題となる．病初期より冠動脈病変を合併していない症例は，発症後約 10 年程度の経過観察しかなされておらず，これに関しては十分な evidence は得られていないが，否定的な見解が大勢を占める．しかし，川崎病による後遺症としての冠動脈病変が残存した症例では遠隔期においてもその病変部位に軽微な慢性持続性血管炎が存在するとの報告[19]が散見され，残存した冠動脈病変部位を中心に動脈硬化が惹起されやすい状態にあることが推察される．

1. 動脈硬化病変の検討

血管内エコーや MRI などの画像診断の進歩により冠動脈壁の形態を中心に動脈硬化病変の検討が比較的容易に行われるようになった．冠動脈障害が認められなかった群では，血管壁の肥厚は観察されていないが，一過性の拡大後の退縮例も含め，冠動脈に拡張病変があった症例では，同部位に内膜，中膜の肥厚が存在し，動脈硬化の存在が示唆され[20]，さらに冠動脈瘤の組織所見においては進行した粥状動脈硬化病変が観察されている[21]．一方，冠動脈病変を有した症例における病変部以外の冠動脈での病理組織学的所見は明確には示されていないので，それらの部位での動脈硬化の有無を論ずることはできない．なお，川崎病における冠動脈瘤の形成部位と成人領域での動脈硬化病変の初発部位はほぼ一致してお

表6　冠動脈外科治療前後の血行動態指標の変動

治療法	症例数	APV		CFR		Shear stress		FFRmyo		血管抵抗	
		術前	術後	術前	術後	術前	術後	術前	術後	術前	術後
CABG 群	10	9.1±1.3	53.6±3.5*	1.12±0.22	2.33±0.16*	11.2±3.6	48.6±13.4*	0.59±0.06	0.92±0.09*	4.1±0.9	1.3±0.8*
縫縮術群	6	9.8±1.7	52.8±3.9*	1.13±0.16	2.26±0.13*	6.2±2.9	38.9±10.8*	0.69±0.10	0.88±0.08*	4.2±0.6	1.4±0.5*

*$p<0.05$ vs. 術後

り，たとえ，瘤以外の部位は問題ないとされたとしても，瘤の部分に存在する動脈硬化性病変を初発部位として，徐々に他の冠動脈壁に拡大していく可能性は否定できない．

2. 血管内皮機能の検討

血管内皮機能の低下と動脈硬化は同義ではないが，内皮機能の低下は動脈硬化の存在を強く示唆する．アセチルコリン，硝酸イソソルビドなどの血管拡張薬負荷前後での血管径や血流量の変化が検討されている．侵襲的方法としては，冠動脈造影，冠動脈内エコー，flow wire などを用いて，また，非侵襲的方法としてはポジトロンCT(PET)や，上腕動脈の血管拡張能(flow-mediated vasodilatation: FMD)などの方法を用いて検討されている．PETの評価では，冠動脈障害の合併の有無を問わず，内皮機能は低下しているとの報告[22]もあるが，一過性拡大後の退縮例を除き，冠動脈に障害が認められなかった症例の内皮機能は大方正常である[20, 23, 24]．最近，動脈瘤の消退後，何年も経過した後に，急性心筋梗塞を併発したとの症例報告が散見される[25, 26]．従って，一旦認められた，冠動脈の拡張性病変を有する症例においては，たとえ画像上冠動脈内腔は正常化したといっても，動脈硬化は発症し，さらにその進展に伴い，成人期以降に虚血性心疾患が惹起される可能性を内在していることを念頭に置くべきである．

一方，冠動脈障害が残存した症例では血管内皮細胞の機能は有意に低下し，動脈硬化の合併を示唆している[20, 23]．さらに，動脈瘤を合併したような重症例では，病変部以外でも血管内皮機能障害の存在が示唆され[23, 27]，動脈硬化を合併している可能性が危惧される．ただし，冠動脈病変以外の血管の動脈硬化の合併については更なる詳細な検討が必要である．

また，川崎病では冠動脈障害が重いほど狭窄性病変に進行していく頻度が高いことも報告されている[28]．狭窄性病変の進行だけでも虚血性心疾患を合併する可能性が高くなるのに，そこに，動脈硬化が加味されることにより，より重症化することが懸念される．

V. 心筋虚血の責任冠動脈に対する外科的治療前後の冠循環動態の評価

APV，CFR，shear stress，FFRmyo，末梢血管抵抗の各指標を用いて，冠動脈外科治療前後での冠血行動態を評価した．

1. CABG 術前後の冠循環動態

CABG(coronary bypass graft)術10例における術前後の評価を行った．術前の冠血行動態はAPV，shear stress，FFRmyoの有意の低下，および末梢血管抵抗が有意に増加しており，心筋への灌流血流量の低下，予備能の低下，さらに末梢循環障害が示唆された[29]．当院ではCABG術を施行する際にnative flowは残存させる術式を取っている．術後もnative flowが残存している症例を対象にバイパス血管ではなくnative flowの認められる血管よりワイヤーを進め，吻合遠位部の冠循環動態の評価を行った．画像上，全例においてCABG吻合部における狭窄性病変の出現は認められなかった．さらに，機能的評価においても，血流パターン，APV，CFR，shear stress，FFRmyo，末梢血管抵抗のいずれにおいても改善が認められ，的確なCABG術により冠循環動態の改善がもたらされることが確認できた(表6，図3)．

2. 縫縮術前後の冠循環動態

当院では有意な狭窄性病変を合併していない巨大冠動脈瘤を有する症例でその末梢に心筋虚血が認められ，かつ，川崎病発症より数年以内で，病変部位に有意な石灰化を伴っていない症例に対し，冠動脈瘤の縫縮術を施行している．有効な縫縮により，血流速度を増加させることによりshear stressが改善し，それに伴い血管内皮機能が改善し，血栓形成が阻止され，ワーファリンから離脱することが可能となる．CABG術と同様に術前には心筋への灌流血流量の低下，予備能の低下，さらに末梢循環障害が示唆された症例でも，十分な縫縮術が施行できた場合には，冠循環動態が改善され，症例によってはワーファリンからの離脱も可能となった(表6，図4)．

VI. まとめ

川崎病に合併する冠動脈瘤内，特に巨大冠動脈瘤内では血流は乱流となり，shear stressは有意に低下し，血管内皮機能の低下が示唆された．また，巨大瘤の末梢部では有意な狭窄性病変を合併していなくてもshear stressの低下，CFRの低下，末梢血管抵抗の増加が認められ，血管内皮機能障害，心筋虚血，さらに微小冠循環障害の存在が示唆された．一方，有意な狭窄性病変の末梢部位で

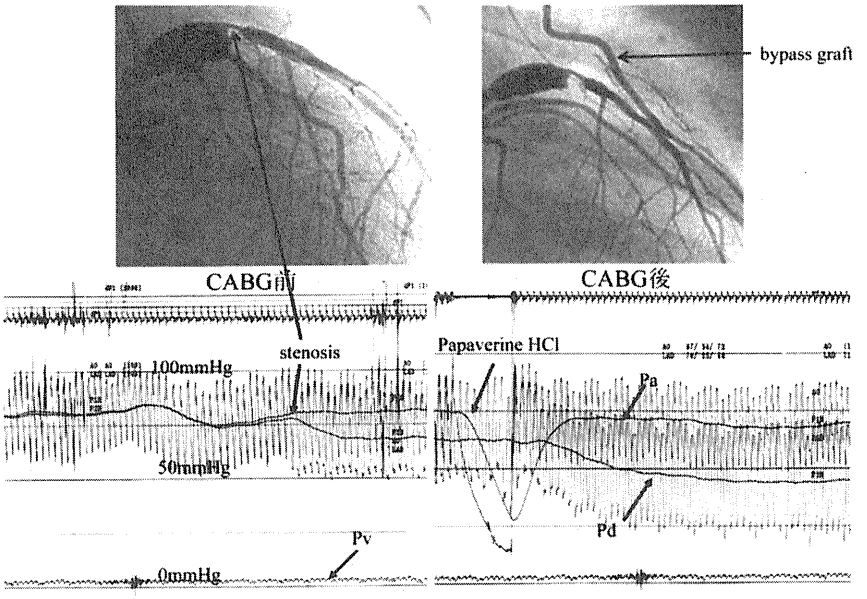

図3　CABG 術前の FFRmyo を用いた狭窄病変の重症度の評価
6 歳男児，1 歳児に川崎病に罹患．瘤遠位部に比較的長い約 90%の狭窄性病変が認められ，左室の前・中隔壁に心筋虚血が認められた．FFRmyo は 0.63 と異常値を呈した．CABG 術の適応と判断し，LITA-LAD のバイパス術を施行．術後の FFRmyo は 0.94 と改善．
CABG: coronary bypass graft, LITA: left internal thoracic artery, LAD: left anterior descending artery

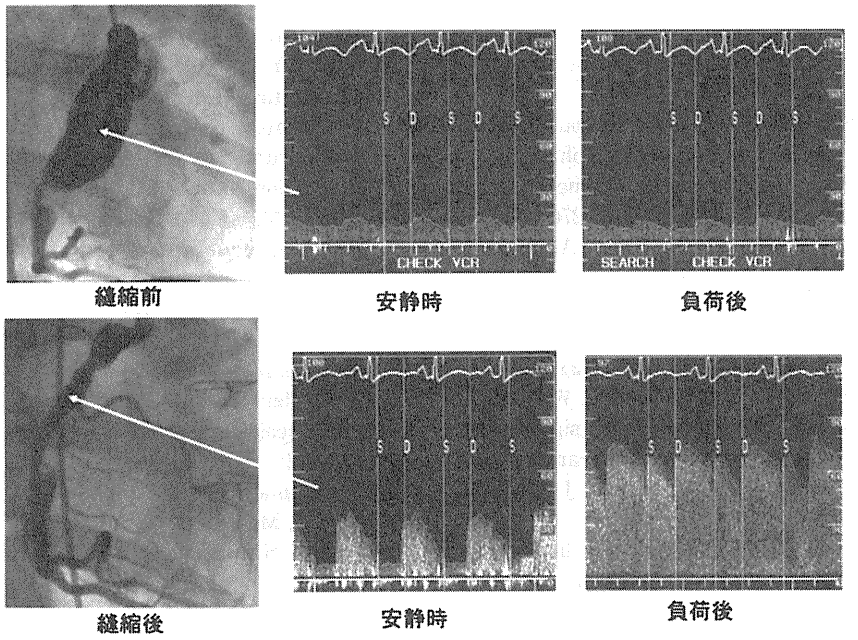

図4　冠動脈瘤縫縮術前後の血流パターン，APV，CFR の変動
A：右冠動脈瘤，最大径は 14 mm で APV は 10 cm/sec，CFR は 1.0，血流パターンは乱流パターン．
B：縫縮術を施行し，瘤の最大径は 5 mm に縮小．それに伴い APV は 20 cm/sec，CFR 3.2，血流パターンも拍動流パターンとすべて正常に復し，ワーファリンより離脱可能となった．

は灌流血液量は減少し，内皮機能障害，心筋虚血が惹起され，さらに灌流圧の低下を凌駕する灌流血液量の低下が起こり，末梢の血管抵抗は上昇することが推察された．このような冠動脈病変の血流動態特性を評価するには画像診断だけでは不十分であり，ドップラワイヤー，プレッシャーワイヤーより得られる血流波形のパターン，APV，CRF，shear stress，FFRmyo，末梢血管抵抗などの諸指標を用いることにより，より正確に評価することが可能と考える．

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ORIGINAL ARTICLE
Pediatric Cardiology and Adult Congenital Heart Disease

Dynamics of Reactive Oxygen Metabolites and Biological Antioxidant Potential in the Acute Stage of Kawasaki Disease

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Background: The dynamics of oxidation/reduction control system activities using reactive oxygen metabolites (ROM) and biological antioxidant potential (BAP) in acute stage patients was evaulated to understand the mechanism of vascular injury and remodeling in Kawasaki disease (KD).

Methods and Results: ROM, BAP, high-sensitivity C-reactive protein (hs-CRP), interleukin-1,2,6, and tumour necrosis factor-α in 19 KD patients were measured. ROM decreased in good correlation only with hs-CRP ($P<0.05$) at 2 weeks after intravenous immunoglobulin (IVIG). Patients were further classified as responding well (Group A) or responding poorly (Group B) to IVIG. Both treatment groups had significantly higher ROM values than the control group ($P<0.01$). ROM decreased in Group A both immediately and 2 weeks after the IVIG treatment ($P<0.05$), but it did not decrease in Group B until 2 weeks post-treatment ($P<0.01$). BAP levels were unremarkable in Group A, but were significantly lower in Group B than in both other groups ($P<0.05$). BAP increased in Group A 2 weeks after IVIG treatment ($P<0.01$), but remained low in Group B ($P<0.01$).

Conclusions: Acute stage KD patients suffer from obvious hyperoxidant stress, and improved in response to IVIG treatment in most patients. Blood BAP level might be a useful index for predicting responsiveness to IVIG the treatment. (*Circ J* 2011; **75:** 2453–2459)

Key Words: Biological antioxidant potential; Intravenous immunoglobulin; Kawasaki disease; Reactive oxygen metabolites; Vasculitis

Kawasaki disease (KD) frequently occurs in infants whose main clinical condition is pan-angitis in the medium- and small-sized arteries throughout the entire body.[1–4] Although the pathology of this condition has not yet been clarified, it appears that vasculitis occurs as a complication of systemic infection.[5]

A key component of the infection process is oxidative stress, which occurs when there is a disruption in the balance between the production and elimination of reactive oxygen species (ROS), resulting in the accumulation of excess in the body.[6,7] Because oxidative stress was recently shown to play a role in amplifying inflammation during the progression of arteriosclerosis to more advanced stages,[8,9] it has been hypothesized that oxidative stress might also be related to the acute and chronic vascular disorders associated with KD (eg, KD vasculitis). KD vasculitis is marked by the presence of inflammatory cells[10] and various cytokines/chemokines in lesions.[11–15] This appears to be part of a vicious cycle in which pan-angitis develops (the acute stage of KD), thus activating inflammatory cells and causing the production of ROS. These cannot be processed by the innately-equipped ROS elimination mechanism, and therefore stimulate the spread of inflammation via the production of various cytokines and the expression of adhesion factors, further stimulating ROS generation. Evidence of this cycle is the increase in 8-isoprostane, a marker of oxidative stress, in the urine of patients in the acute and distal stages of KD.[16–18] However, reports of this pattern have been inconsistent, and the clinical significance of this result has not yet been verified.

Further, previous studies have focused only on oxidative stress levels, while ignoring the activity of the body's natural ROS elimination system. It is important to consider both of these in tandem, as the oxidation/reduction control system has evolved specifically to maintain homeostasis in response to

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Table 1. Information of Patients (n=19)

Patient no.	Responsibility to IVIG	Gender	Age (months)	The beginning day of treatments	Coronary lesions	Dilatation	Additional treatment	Severity of illness index (γ-score)
1	Favorable	F	70	5	–	–	–	0
2	Favorable	F	22	5	–	–	–	3
3	Favorable	F	24	4	–	–	–	3
4	Favorable	M	12	4	–	–	–	3
5	Favorable	M	11	5	–	–	–	1
6	Favorable	M	17	4	–	–	–	Unknown
7	Favorable	M	18	3	–	–	–	2
8	Favorable	M	26	5	–	–	–	2
9	Favorable	M	14	3	–	–	–	2
10	Favorable	F	23	6	–	–	–	0
11	Favorable	M	11	3	–	–	–	3
12	Favorable	F	11	9	–	–	–	1
13	Favorable	F	39	4	–	–	–	3
14	Unfavorable	M	24	3	○	○	IVIG+PSL	6
15	Unfavorable	M	10	4	–	–	IVIG+PSL	5
16	Unfavorable	F	28	4	–	–	IVIG+PSL	6
17	Unfavorable	M	24	4	–	–	IVIG+PSL	6
18	Unfavorable	F	36	5	○	○	IVIG+PSL	6
19	Unfavorable	F	10	3	–	–	IVIG+PSL	5

IVIG, intravenous immunoglobulin; M, male; F, female.

systemic challenges. To more fully investigate these complex dynamics, we measured levels of reactive oxygen metabolites (ROM) and biological antioxidant potential (BAP) in the blood of 19 patients receiving treatment for acute stage KD. These measurements were designed to evaluate oxidative stress levels and the activity of the ROS elimination system, respectively, in immunocompromised individuals.

In addition, having used ROM and BAP for this oxidation stress evaluation has a significant meaning. Up to now, the measurement of the oxidant stress was not easy. Electron spin resonance (ESR) method as a golden standard of the free radical measurement requires skill and expertise because of the use of an extremely complex technique, and the measurements of various oxidative-stress markers already known are time-consuming because of their use of immunological methods. Therefore, there were a lot of problems in clinical use although they were useful for the investigative purpose. However, the measurement of ROM and BAP is very handy. We can get both results in 10 min and it shows excellent reproducibility. To sum up, handiness, promptness, and reproducibility are all factors that are indispensable characters in the clinical application. In this respect, we can estimate high usefulness of the procedure that makes a clear distinction with the current oxidation stress evaluation.

Methods

This study focused on 19 patients (**Table 1**) with acute KD (median age: 1.8 years, range: 0.8–5.8 years) and 7 control subjects (median age: 1.1 years, range: 0.8–6.3 years). All patients were diagnosed with KD according to the diagnostic standard of the Japanese Society of Kawasaki Disease (JSKD).[19] As recommended by the JSKD, patients were treated with intravenous immunoglobulin (IVIG; 2 g/kg single administration) and oral aspirin ($30\,mg \cdot kg^{-1} \cdot day^{-1}$).[20] Blood samples were collected immediately before, immediately after, and 2 weeks after the IVIG treatment so that ROM, BAP, high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-1,2,6, and tumor necrosis factor (TNF)-α levels could be measured. Blood samples were also collected from control subjects, for comparison. When possible, blood sampling was performed in the early morning, after patients had fasted overnight.

Thirteen of the patients responded well to treatment (Group A). The remaining 6 patients continued to experience a fever of 37.5°C or more during the 48 h following the initial IVIG administration, and required additional IVIG or steroid treatment (Group B). However, the fever had decreased to 37.5°C or below by the time of the final blood sampling.

All study protocols were approved by the ethical committee at the Kyoto Prefectural University of Medicine Graduate School of Medical Science. Informed written consent was obtained from the parents of all study subjects and particular care was taken to protect human rights and personal information.

Measuring ROM and BAP Levels

Levels of both ROM and BAP were measured with a Free Radical Elective Evaluator (FREE®; Wismerll Co Ltd, Tokyo, Japan).[21–23] Measurement of ROM levels is based on the ability of transition metals to catalyze, in the presence of peroxides, the formation of free radicals trapped by an alchilamine. The alchilamine reacts to form a colored radical that is detectable at 505 nm. The results are expressed in conventional units called U.CARR (Carrtelli units), such that 1U.CARR corresponds to 0.8 mg/L H_2O_2. To measure BAP, blood samples [BP (e-), containing the electron-donating, antioxidant blood plasma barrier molecule] were added to a colored solution containing ferric chloride ($FeCl_3$; a source of ferric ions, Fe^{3+}) bound to a chromogenic substrate (AT, a derivative of thiocyanate). This caused the Fe^{3+} to be reduced to ferrous ions (Fe^{2+}), leaving oxidized blood plasma barrier molecules (BP) and a decolored solution:

Table 2. Change Rate of Each Parameters (Measurements)

	Pre-IVIG	Immediately after IVIG	2 weeks after IVIG
ROM			
Change rate	100	93.6 (101.3/83.5)	70.6 (78.1/64.9)
Value	615 (668/539)	555 (638/485)	430 (492/372)
BAP			
Change rate	100	100.5 (105.9/93.9)	106.4 (110.4/99.8)
Value	2,555 (2,778/2,460)	2,552 (2,850/2,377)	2,719 (3,000/2,638)
hs-CRP			
Change rate	100	63.0 (87.1/45.7)	0.8 (2.2/0.5)
Value	6.6 (12.3/4.3)	3.7 (8.1/2.6)	0.07 (0.10/0.04)
IL-1			
Change rate	100	67.9 (110.2/ 56.7)	67.2 (104.5/51.2)
Value	240 (317/174)	169 (237/138)	163 (231/146)
IL-2			
Change rate	100	63.0 (116.5/48.0)	61.3 (97.3/43.6)
Value	596 (1,054/387)	342 (616/265)	344 (516/268)
IL-6			
Change rate	100	14.8 (63.0/7.3)	9.3 (14.0/5.8)
Value	2,768 (4,821/1,279)	395 (558/253)	221 (305/184)
TNF-α			
Change rate	100	77.7 (112.2/65.5)	82.7 (121.9/57.1)
Value	245 (349/182)	219 (255/166)	216 (242/182)

Each number show median and (upper/lower quartile).
ROM, reactive oxygen metabolites; BAP, biological antioxidant potential; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; TNF, tumor necrosis factor. Other abbreviation see in Table 1.

Table 3. ROM and BAP Levels in Subjects in the Control (n=7) and Treatment Groups (n=13, Group A; n=6, Group B), Immediately Before, Immediately After, and 2 Weeks After IVIG Treatment

	ROM (U.CARR)			BAP (mol/L)		
	Pre-IVIG	Post-IVIG	2 weeks after	Pre-IVIG	Post-IVIG	2 weeks after
Favorable response (n=13)						
Median	633	555	466	2,705	2,714	2,778
Upper/lower quartile	687/555	646/478	506/381	2,883/2,510	2,940/2,462	3,015/2,705
Unfavorable response (n=6)						
Median	564	557	381	2,474	2,438	2,622
Upper/lower quartile	611/531	610/483	423/304	2,557/2,413	2,626/2,313	2,703/2,380
Control (n=7)						
Median		312			2,674	
Upper/lower quartile		327/300			2,745/2,572	

Abbreviations see in Tables 1,2.

1. $FeCl_3$+AT (uncolored)→$FeCl_3$–AT (colored)
2. $FeCl_3$–AT (colored)+BP (e-)→$FeCl_2$+AT (uncolored)+BP.

The chromatic change that results from this process can be measured by with a photometer at 505 nm, and is directly proportional to the ability of the plasma to reduce ROS. Ten-microliter blood sample aliquots were required from each patient for these protocols. The samples were mixed with the colored solution and incubated for 5 min at 37°C prior to photometric analysis. The pH of the $FeCl_3$ – AT solution was 2.3 and it remained in the range of 2.3–2.4 throughout the procedure.

Statistical Analysis

Data values are presented as medians and interquartile ranges because almost all the values are not normally distributed. All data were analyzed using SPSS software version 11.0 (SPSS Japan Inc, Tokyo, Japan). Paired t-tests or Mann-Whitney U-tests were used to make comparisons between groups. Significance was defined as $P<0.05$.

Results

Change of Each Parameter

We measured each parameter in all patients and calculated the change rate by assuming the value at pre-IVIG to be 100 (Table 2). We examined whether there was a correlation in the change of the oxidant stress and that of hs-CRP and the proinflammatory cytokine.

The levels of ROM and BAP did not show the correlation with neither hs-CRP nor the proinflammatory cytokines in their variation from pre-IVIG to immediately post-IVIG. The ROM showed the correlation only hs-CRP, although BAP did

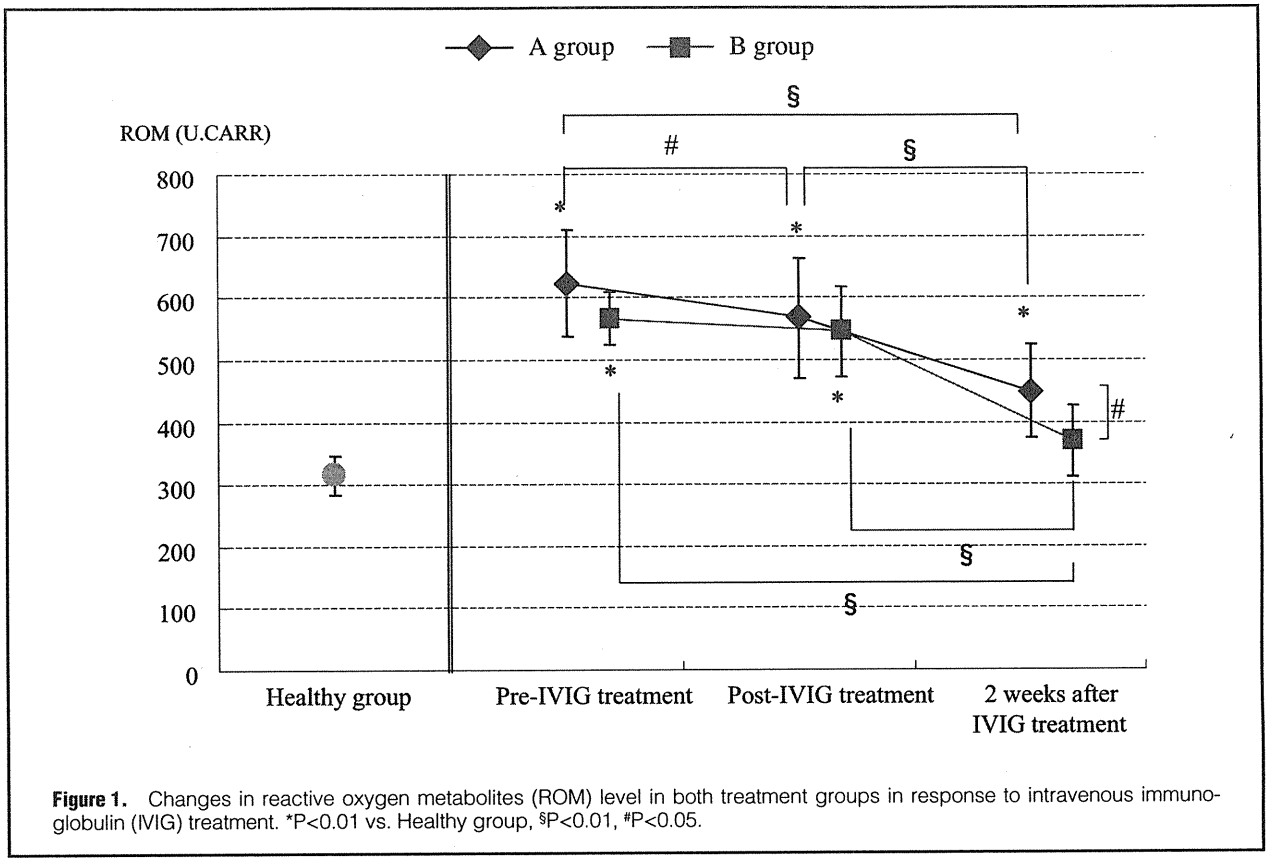

Figure 1. Changes in reactive oxygen metabolites (ROM) level in both treatment groups in response to intravenous immunoglobulin (IVIG) treatment. *P<0.01 vs. Healthy group, §P<0.01, #P<0.05.

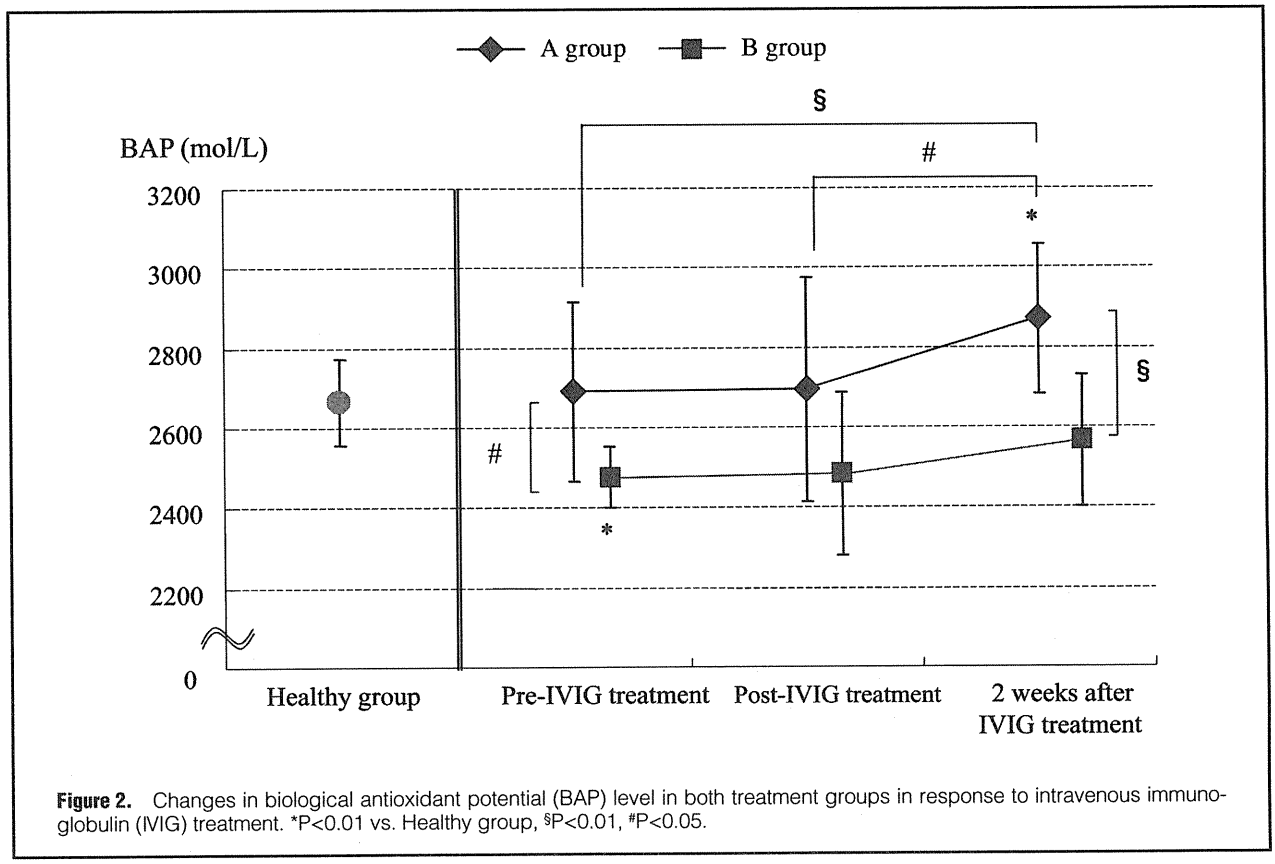

Figure 2. Changes in biological antioxidant potential (BAP) level in both treatment groups in response to intravenous immunoglobulin (IVIG) treatment. *P<0.01 vs. Healthy group, §P<0.01, #P<0.05.

not show the correlation with all parameters in their variation from immediately after IVIG to 2 weeks after IVIG ($P<0.05$).

ROM and BAP Levels in the Control Group

In the control group, the ROM level was 312 (327/300) (median (upper/lower quartile U.CARR) and the BAP level was 2,674 (2,745/2,572) (mol/L) (**Table 3**). These values are slightly higher than those that have previously been reported as adult standard levels (ROM: 200–300 U.CARR; BAP: >2,200 mol/L).[24]

Dynamics of ROM During the Acute Stage of KD

Immediately before IVIG, the ROM level were significantly higher in Group A than in the control group (633 (687/555) U.CARR; $P<0.0001$) (**Table 3**; **Figure 1**). However, ROM rapidly declined to significantly lower levels both immediately after (555 (646/478) U.CARR; $P<0.05$ and 2 weeks after (466 (506/381) U.CARR; $P<0.01$) the IVIG treatment.

Immediately before the IVIG treatment, the ROM level was also significantly higher in Group B than in the control group (564 (611/531) U.CARR; $P<0.01$). The IVIG treatment did not facilitate any immediate declines in ROM levels among Group B patients (557 (610/483) U.CARR, NS). After 2 weeks, however, ROM was significantly lower than it had been immediately before the treatment (381 (423/304) U. CARR; $P<0.01$).

It is important to note that ROM level was statistically similar in Groups A and B immediately before the treatment. Thus, the different responses to the IVIG treatment were not caused by differences in levels of ROS.

Dynamics of BAP During the Acute Stage of KD (Figure 2)

In Group A, BAP did not change significantly from immediately before the IVIG treatment (2,705 (2,883/2,510) mol/L) to immediately afterward (2,714 (2,940/2,462) mol/L). However, BAP was obviously higher 2 weeks after the treatment (2,778 (3,015/2,705) mol/L). This was significantly higher than both the baseline value ($P<0.01$) and that recorded immediately after the treatment ($P<0.05$).

Immediately before the IVIG treatment, BAP was significantly lower in Group B than in both the control group ($P<0.01$) and in Group A ($P<0.05$). Unlike the pattern observed in Group A, BAP levels in Group B did not change significantly from immediately before the treatment (2,474 (2,557/ 2,413) mol/L), to immediately after the treatment (2,438 (2,626/2,313) mol/L), to 2 weeks after the treatment (2,622 (2,703/2,380) mol/L).

We did not perform multiple regression analysis because we could not find any significant variable excluding BAP in univariate analysis. Furthermore, only BAP was still selected although the model for the multivariate analysis. The area under the receiver operating curve is 0.80 (95% confidence interval 0.59–0.99).

Discussion

Here, we found that the reduction of ROM from immediately after the IVIG to 2 weeks after IVIG correlated to the movement of hs-CRP, which is a general inflammatory marker. The fact might suggest their close relationship between the oxidative stress and inflammation. Furthermore, we found that patients with acute stage KD have abnormally high levels of ROM in their blood, indicating an increased production of ROS. When given an IVIG treatment, some patients experienced ROM reductions, while others did not, suggesting that this treatment will not always be effective in suppressing ROS production.

Baseline BAP levels were either similar to (Group A) or lower than (Group B) those in control individuals. Either way, this indicates that the KD patients had not launched an endogenous antioxidant response. However, patients in Group A experienced clear increases in BAP 2 weeks after receiving IVIG, demonstrating the effectiveness of this treatment in stimulating antioxidant activity. The pattern of change in BAP level was similar among patients in Group B, but their absolute BAP values were significantly lower than those in both control individuals and IVIG-treated patients in Group A. Again, this indicates that while the IVIG treatment is effective against acute KD symptoms, it cannot be relied upon to work equally well in all cases.

In cases of KD-associated vasculitis, cytokines such as TNF-α and IL-6 increase during the acute stage in response to the release of various prophlogistic substances from the infiltrated monocyte/macrophage.[11–15] The released TNF-α induces vascular endothelial cells to express adhesive factors that prime neutrophils and monocytes. Furthermore, it also acts on endothelial cells and fibroblasts, induces various chemokines, facilitates migration of inflammatory cells to the inflammatory site, and increases production of cytokines such as IL-6, thus increasing inflammation. NAD(P)H oxidase is then activated in the inflammatory cells (eg, neutrophils and macrophages) that were primed by the inflammatory cytokines; this leads to the rapid production of a large amount of ROS.[25] Inducible nitric oxide (NO) synthase in the inflammatory cells produces NO, an unstable radical that changes to peroxynitrite (ONOO-) when exposed to ROS. Peroxynitrite is extremely responsive, and its strong oxidation activity is capable of directly disabling vascular tissues.[26] We suspect that this might be an important element driving the progression of vascular disorders associated with KD. NAD(P)H oxidase[27] on the endothelial cell membrane also reacts with the xanthine oxidase system in vascular endothelial cells[28] and TNF-α in the blood, and causes the activation of the arachidonate cascade, which generates prophlogistic substances such as leukotriene[29] and produces ROS as a by-product of metabolism.

The ROM measured in this study is a generic name for organic molecules that have been oxidized by ROS such as hydroperoxide (R-OOH). One important ROM is hydroxyperoxide, which is produced by the oxidization of physiologically vital organic molecules such as lipids, proteins, and nucleic acids. The presence of hydroxyperoxide is considered an excellent marker of oxidative damage,[30–33] and can easily be measured by the FREE® system used in this study. Results from this method correlate highly with those produced using ESR methodologies.[34,35] The patterns of ROM decrease shown in both treatment groups here are not surprising, given what is currently known about KD and the inflammation pathway. The more novel result of this work is the discovery that ROS increased so rapidly. Additionally, our techniques here have shown that the ROM measurement is useful in clinical situations for assessing inflammatory dynamics in lesions, as well as investigating the efficacy of inflammation alleviation treatments. Further, our results clearly demonstrate that the IVIG inflammation alleviation treatment cannot be relied upon to significantly reduce inflammation in all patients, although it does reduce ROS production more often than not (eg, in 13 of 19 patients).

There are also homeostasis mechanisms that combat the presence of ROS by eliminating these elements in order to maintain the balance between oxidization and antioxidization.[36,37] Antioxidants have both endogenous (eg, albumin,

transferrin, ceruloplasmin, bilirubin, ureic acid, reduced glutathione, etc) and exogenous (eg, tocopherol, carotin, ubiquinone, ascorbic acid, methionine, flavonoid, polyphenol, etc) origins. The FREE® system, used here to measure the comprehensive antioxidative potency in patients' blood, is based on the same principle as the ferric reducing ability of plasma assay method,[38] which has widely been recognized as an effective measurement technique.

Here, we found that changes in BAP in acute KD patients did not mirror changes in ROS. Furthermore, BAP activity was clearly lower in some patients (Group B) than in others (Group A), even after the IVIG treatment. Oxidative stress alleviation was delayed in Group B, and we hypothesize that this led to a build-up of abnormal ROS, leading to further increases in oxidative stress and additional inflammation, via a positive feedback loop. This phenomenon might be one factor leading to the higher rate of coronary arterial disorders in IVIG-unresponsive cases.[39,40]

The results of our work also suggest that baseline BAP levels can be used to predict whether patients will respond well to IVIG treatment and to assess the clinical conditions of vasculitis. Previously, it has been shown that prolonged inflammation, such as that likely present in Group B, increases the rate at which patients suffer coronary arterial complications.[41] Therefore, it is important to select an effective initial treatment in order to decrease inflammation as early and as rapidly as possible. This can be facilitated via the use of an appropriate biomarker that can be used as an index of the severity of inflammation. Our work indicates that BAP levels, either before or during treatment, can be used for this purpose. Moreover, BAP measurements can be performed within a few minutes following sample collection, which further enhances the clinical usefulness of this modality.

Although we have not directly shown a link between the clinical conditions of KD and activity of the oxidation/reduction control mechanism, we believe there is good evidence suggesting that the two are closely related: In patients with KD-associated coronary arteritis, inflammatory cells such as macrophages remain in place for approximately 2–3 months after infiltration. Since these macrophages are producing ROS, the coronary arteries are exposed to excessive oxidative stress for long periods of time. Thus, in patients who respond to IVIG treatment by upregulating their antioxidant activity, vascular disorders should be shorter-lived and vascular remodeling should proceed at a higher rate, than among patients who do not respond to the IVIG treatment. In order to investigate this hypothesis, it will be important to plot ROM and BAP values, as well as document clinical conditions, of a large study group over a long study period.

Further study is needed to make this research results more significant because the number of cases is still little in this study. The relationship between the antioxidative potency and the IVIG reactiveness obtained this time is a very intriguing result. We think that further examination leads to the elucidation of the cause of KD and the proposal of new treatment methods.

Conclusion

We have shown that patients with acute stage KD suffer from increased levels of oxidative stress, which, in most cases, can be reduced via the anti-inflammatory activities of IVIG. We also found that antioxidant activities increase slowly, relative to changes in ROS levels. Nearly a third of patients examined here responded poorly to the IVIG treatment, and these individuals had lower initial BAP levels than either controls or IVIG-responsive patients. This suggests that BAP can be used to predict the likelihood that anti-inflammatory treatments will be effective in KD patients. The ROM measurement, also, might be useful in a clinical setting, for evaluating inflammatory dynamics in lesions and tracking the effectiveness of anti-inflammatory treatments.

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Disclosures

None.

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ORIGINAL ARTICLE

Serum adipokine profiles in Kawasaki disease

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Abstract Adipokines are cytokines derived from adipose tissue. Recently it has been established that adipokines are closely linked to the pathophysiology of not only metabolic diseases, such as diabetes mellitus, obesity, and atherosclerosis, but also to inflammation and immune diseases. In this study we measured serum levels of adipokines in patients with acute Kawasaki disease to investigate the role of adipokines in the pathophysiology of Kawasaki disease. Serum resistin, high-molecular-weight (HMW) adiponectin, leptin, and visfatin levels were measured by enzyme-linked immunosorbent assay in a total of 117 subjects: 56 patients with acute Kawasaki disease, 30 healthy children, and 31 patients with acute infectious diseases. Serum resistin levels in patients with Kawasaki disease were significantly higher than those of healthy children and patients with acute infectious diseases. In contrast, mean serum HMW adiponectin, leptin, and visfatin levels in patients with Kawasaki disease exhibited no statistically significant differences compared with those in healthy children and patients with infectious diseases. Serum resistin levels decreased significantly after administration of intravenous immune globulin. Serum resistin levels on admission were significantly higher in nonresponders compared with responders to intravenous immune globulin therapy. A multivariate model revealed that C-reactive protein was a factor that was significantly related to elevated serum resistin level in patients with Kawasaki disease. In patients with Kawasaki disease, serum resistin levels were elevated, but decreased to nearly normal after intravenous administration of immune globulin. In contrast, serum HMW adiponectin, leptin, and visfatin levels showed no statistically significant changes. These findings suggest that resistin plays an important role, while other adipokines do not play a major role, in the pathogenesis of Kawasaki disease.

Keywords Adipokines · Resistin · C-reactive protein · Kawasaki disease

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Introduction

Kawasaki disease is a systemic vasculitis of childhood that was first reported by Tomisaku Kawasaki in 1967 [1]. Patients manifest with fever, bulbar conjunctival injection, changes of the oropharyngeal mucosa, changes of the peripheral extremities, cervical lymphadenopathy, and polymorphous rash [2]. In Japan there are approximately 10,000 new patients annually [3]. The most important complication of this disease is development of coronary lesions that result in acute myocardial infarction. Intravenous immune globulin is a standard therapy that is effective in about 70% of patients, but the cause of the disease has been unclear.

Adipokines or adipocytokines including resistin, adiponectin, leptin, and visfatin are bioactive molecules that are produced and secreted by adipose tissue [4]. Adipokines have various actions in the human body that regulate metabolic conditions, and may have a central role in regulation of insulin resistance [5, 6].

Resistin is an amino acid peptide that belongs to a cysteine-rich secretory protein family [7]. Circulating resistin levels are elevated in humans by obesity and diabetes [8]. Resistin levels are also associated with increasing coronary artery calcification and are predictive of coronary atherosclerosis [9]. Adiponectin is a 244-amino-acid polypeptide that has three isoforms: low molecular weight, middle molecular weight, and high molecular weight (HMW). Decreased levels of HMW adiponectin are associated with coronary artery disease and type 2 diabetes [10, 11]. Leptin is a protein of 167 amino acids. Circulating leptin levels reflect adipose tissue mass, and hyperleptinemia is associated with obesity and other metabolic diseases [12, 13]. Visfatin is one of the adipokines identified in 2004, being predominantly produced and secreted in visceral fat; its expression level in plasma increases during development of obesity [14].

These adipokines show obviously links to metabolic diseases, however recent studies have also suggested that some adipokines might play a role in inflammation and immune diseases [15]; for instance, we have previously shown that serum levels of resistin, leptin, and adiponectin were all associated with C-reactive protein (CRP) level in patients with rheumatoid arthritis, suggesting that these adipokines may act as proinflammatory cytokines in this disease [16].

The object of this study is to clarify serum levels of resistin, HMW adiponectin, leptin, and visfatin in patients with Kawasaki disease during treatment with intravenous immune globulin, and to evaluate the relationships between serum adipokines and their clinical measures.

Methods

Patients

Fifty-six patients (36 males and 20 females, mean age 29.8 ± 1.7 months) with acute-phase Kawasaki disease who were admitted to our university hospital participated in this study. All patients met American Heart Association diagnostic criteria for Kawasaki disease [2]. These patients were treated with oral aspirin and 1 or 2 g/kg intravenous immune globulin after admission. As controls, we collected serum samples from 30 healthy children and 31 patients with acute infectious diseases (14 patients with pharyngitis, 9 with bronchitis, 5 with gastroenteritis, and 3 with exanthema subitum). The protocol for the study was approved by the Ethics Committee of Toho University Hospital. Informed consent was obtained from the parents of all patients.

Adipokine measurements

Blood samples were collected from the patients with acute-phase Kawasaki disease upon admission (before intravenous immune globulin) and at 24–48 h after intravenous immune globulin treatment. Serum resistin, HMW adiponectin, leptin, and visfatin were measured using enzyme-linked immunosorbent assay (ELISA) kits. Serum resistin and leptin levels were measured in all 56 patients, but HMW adiponectin and visfatin were measured in 38 patients because the volume of serum samples was too small to perform all four analyses. Resistin and leptin ELISA kits were both purchased from B-Bridge International, Inc. (Sunnyvale, CA, USA). ELISA kits for HMW adiponectin and visfatin were obtained from Fujirebio, Inc. (Tokyo, Japan) and Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA), respectively.

Biochemical measurements

All of the patients with Kawasaki disease were examined for complete blood cell counts and serum chemistry, including CRP and electrolytes, before immune globulin therapy. Latex nephelometry (Sekisui Medical Co., Tokyo, Japan) was used for CRP measurement.

Statistical analysis

Comparisons between the three groups were made using the Kruskal–Wallis test. Serum adipokine levels before and after intravenous immune globulin were compared by the Wilcoxon matched-pairs signed-rank test. Correlations between serum adipokines and laboratory data were analyzed by simple linear regression analysis. Multiple regression analysis was used for studying multivariable models. Statistical significance was determined at $p < 0.05$. Statistical analyses of the data were conducted using the StatMate III software program (ATMS, Tokyo, Japan).

Results

Characteristics of the study population

The characteristics of the 3 groups are shown in Table 1. There were no statistically significant differences in age, gender or body weight among the 3 groups of children. In patients with Kawasaki disease, mean ± SD age was

Table 1 Background characteristics of the three patient groups

	Age (months)	Gender (M/F)	Body weight (kg)
Patients with Kawasaki disease ($n = 56$)	29.8 ± 21.7	36/20	12.1 ± 3.6
Patients with acute infectious diseases ($n = 31$)	29.2 ± 17.5	20/11	12.1 ± 3.5
Healthy children ($n = 30$)	26.9 ± 13.0	19/11	11.7 ± 2.4

Values are mean ± SD

Table 2 Clinical characteristics of the patients with Kawasaki disease

	Age (months)	Days on IVIG	Serum CRP conc. (mg/dl)	WBC counts ($\times 10^3/\mu l$)	Sodium conc. (mEq/l)	IVIG responder*	CAL
Male 36	26 ± 18	4.5 ± 1.8	7.2 ± 5.2	14.2 ± 5.2	131.5 ± 2.8	21 (58.3%)	3 (8.3%)
Female 20	37 ± 25	4.9 ± 1.9	4.7 ± 4.6	7.5 ± 3.1	135.5 ± 2.3	17 (85.0%)	1 (5.0%)

Values are mean ± SD or cases (percentages)

IVIG intravenous immune globulin therapy, *CRP* C-reactive protein, *conc.* concentrations, *WBC* white blood cell, *CAL* coronary arterial lesion

* Patients who had cessation of fever (<37.5°C) after IVIG and needed no additional therapy

29.8 ± 21.7 months. The clinical profiles of patients with Kawasaki disease are presented in Table 2. Thirty-eight patients (67.9%, 21 males, 17 females) responded to intravenous immune globulin infusion. Four patients had coronary lesions detected by echocardiography at discharge, even after immune globulin therapy.

Serum adipokine levels in patients with Kawasaki disease

Serum adipokine levels are shown in Fig. 1. Serum resistin levels were significantly higher in patients with Kawasaki disease (mean 31.5 ± 20.0, median 27.5 ng/ml) compared with healthy controls (mean 5.0 ± 6.8, median 3.3 ng/ml, $p < 0.001$) and patients with acute infectious diseases (mean 10.6 ± 9.2, median 6.9 ng/ml, $p < 0.001$). However, serum HMW adiponectin, leptin, and visfatin levels in patients with Kawasaki disease (HMW adiponectin: mean 10.8 ± 5.1, median 10.1 μg/ml; leptin: mean 2.4 ± 4.0, median 1.6 ng/ml; visfatin: mean 11.1 ±5.5, median 9.5 ng/ml) showed no statistically significant differences compared with those in healthy controls (HMW adiponectin: mean 23.5 ±9.9, median 22.7 μg/ml; leptin: mean 2.0 ±0.7, median 1.9 ng/ml; visfatin: mean 14.9 ±15.7,

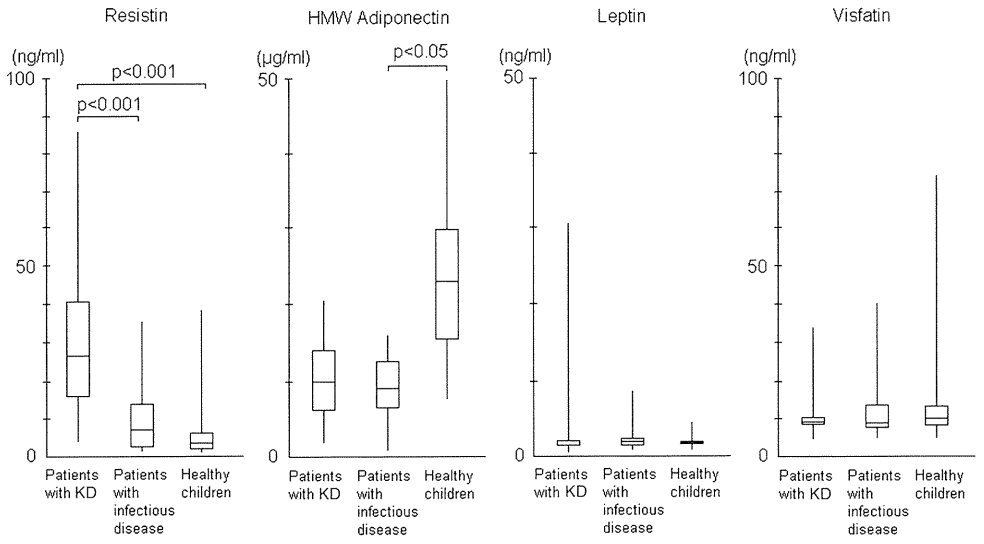

Fig. 1 Serum adipokine levels in the three groups. In the *box plots*, *horizontal lines* indicate median values, and the *lower* and *upper ends* of *boxes* represent the 25th and 75th percentiles. In patients with Kawasaki disease, serum resistin levels were significantly higher than in patients with infectious diseases and in healthy children ($p < 0.001$). Serum high-molecular-weight adiponectin, leptin, and visfatin levels in patients with Kawasaki disease exhibited no statistically significant differences compared with those in healthy controls and patients with acute infectious diseases. *KD* Kawasaki disease, *HMW* high molecular weight