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# DNAの分解異常による関節炎

川根公樹，長田重一

DNAは自己の遺伝情報を担う重要な分子でありながら，生体内ではいくつかの局面で分解される．われわれはDNA分解の生理的意義を解析する過程で，DNaseⅡ遺伝子を欠損したマウスがヒト関節リウマチに類似した関節炎を発症することを見出した．このマウスで観察された病態を示し，DNA分解異常という，これまで考えられていなかった原因で関節炎が発症した機序について考察する．

自己の遺伝情報を担うDNAは，生体内でのいくつかの局面において，積極的に分解される．われわれはDNA分解の生理的意義と分子機構を明らかにする目的で，DNA分解酵素を欠損したマウスを作製してきた[1]．その結果，アポトーシス細胞のDNA分解は，死細胞内で作用するCAD（caspase-activated DNase）と貪食細胞のリソソームで作用するDNaseⅡが協調して担っていることを明らかにした[2]．また，赤血球前駆細胞から脱核時に放出された核はマクロファージに貪食され，この核DNAはDNaseⅡによって分解されることも明らかにした[3]．DNaseⅡ遺伝子が欠損し，DNA分解が妨げられると，マクロファージは未分解DNAを蓄積し，活性化されてインターフェロン（IFN）-βを産生する[4][5]．IFN-βはT細胞や赤血球前駆細胞の増殖，分化を抑制し，その結果，胸腺の小型化や重度の貧血により，マウスは胎生期で死亡する．これらの結果は，プログラム細胞死や赤血球の成熟過程で起こるDNAの分解が，胎生期における血球細胞ひいては個体の正常発生に必須であることを示している．しかし一方，DNaseⅡ遺伝子の欠損は胎生致死をもたらすため，成体マウスでDNaseⅡ遺伝子の生理的意義を解析することは不可能であった．今回われわれは，成体でDNaseⅡ遺伝子を欠損する2つのマウス系統を樹立し，このマウスが関節炎を発症することを見出した．

## DNaseⅡ変異マウスはヒト関節リウマチに類似した関節炎を発症した

われわれは，成体でのDNaseⅡの働きを明らかにする目的で，2つのDNaseⅡ欠損マウスを作製した．1つはIFN-βの受容体，IFN-IR（IFN type I receptor）とDNaseⅡの二重欠損マウスである．二重欠損マウスは貧血を起こさずに誕生し，DNaseⅡ欠損マウスでの致死性貧血の原因がIFN-βであることを証明するとともに，成体におけるDNaseⅡの役割を解析することが可能となった．もう1つの系統としてDNaseⅡコンディショナルノックアウト（KO）マウス（Mx1-Cre：Tg，DNaseⅡ：flox/-）を作製した．このマウスに，IFNを誘導するpoly（I：C）を投与すると，IFN誘導遺伝子であるMx1-プロモーターの支配下にあるCreリコンビナーゼが発現し，不可逆的にDNaseⅡ遺伝子を除去することが可能となる．こ

Chronic polyarthritis caused by undigested mammalian DNA in macrophages

Kohki Kawane/Shigekazu Nagata：Graduate School of Frontier Biosciences, Osaka University（大阪大学大学院生命機能研究科）

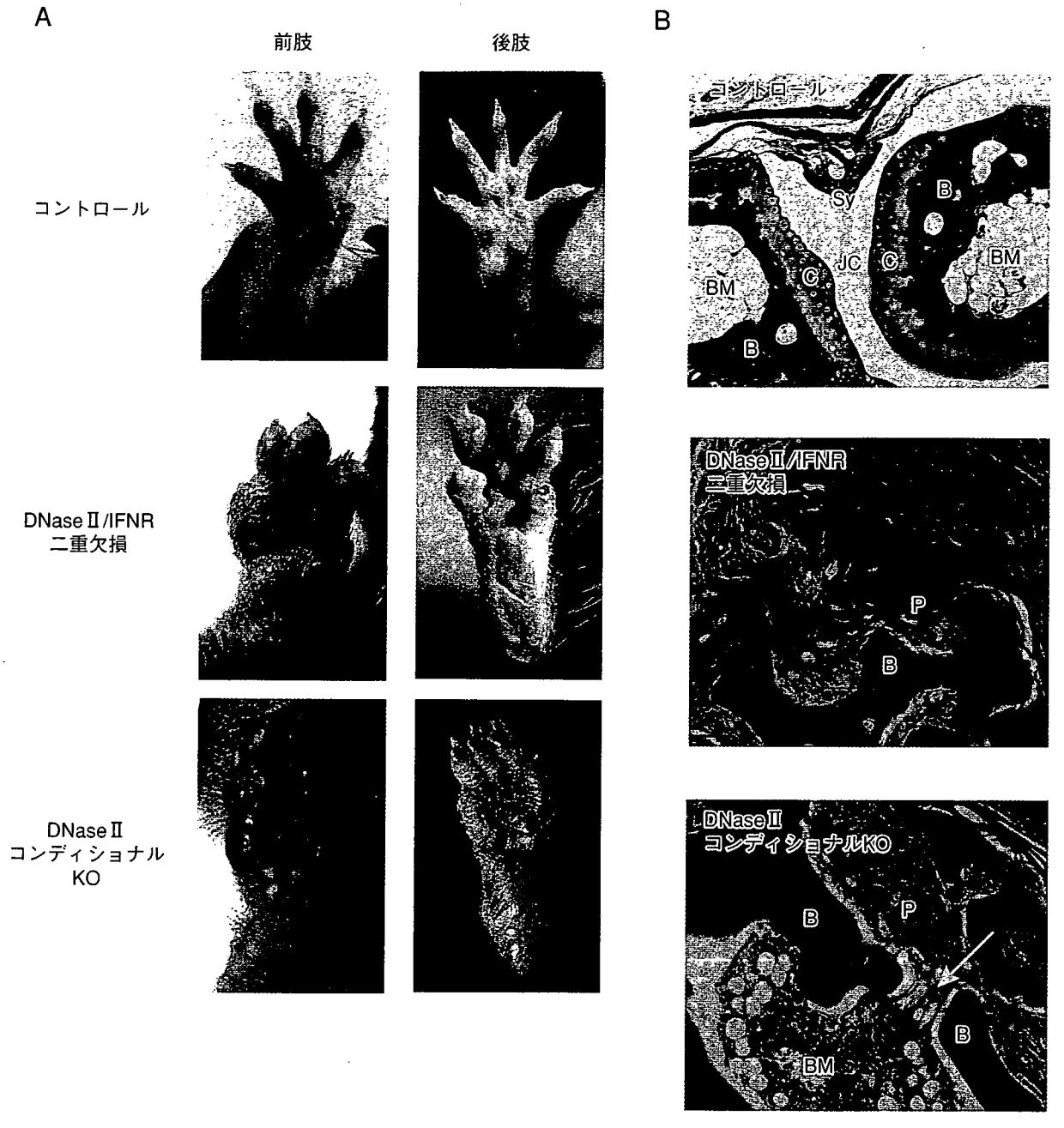


**図　DNaseⅡ変異マウスにおける前肢，後肢のヒトRA様病変**

**A**）DNaseⅡ変異マウスにおける前肢，後肢末端の腫脹と変形．**B**）DNaseⅡ変異マウスにおける関節炎の組織像．関節部位の組織切片のHE染色像を示す．コントロールマウスの正常像では骨の末端に軟骨が存在し，2つの骨がむかいあう形で関節が構成される．骨の間には関節腔とよばれる空洞がみられ，通常，関節液で満たされている．また，関節を覆う形で滑膜が観察される．二重欠損マウス，コンディショナルKOマウスでは関節部で激しい炎症が起きている（本文参照）．矢印：骨髄と骨外部が貫通している部位，B：骨，C：軟骨，BM：骨髄，JC：関節腔，Sy：滑膜，P：pannus．スケールバー＝50 $\mu$m
（文献9より転載）

の2系統のマウスは同様の表現型を示したことより，2系統をあわせてDNaseⅡ変異マウスとよぶ．DNaseⅡ変異マウスは，観察を続けるにつれ，前肢，後肢の末端が腫脹した（図A）．腫脹は加齢とともに増悪化し，指が変形するとともに，関節部の可動性が著しく低下した．この所見は，二重欠損マウスでは生後2～3カ月くらいから観察され，5～6カ月までには全例で腫脹がみられた．コンディショナルKOマウスにおいて

もpoly（I:C）投与後，同様のタイムコースでこの異常が発生し，この異常は，DNase Ⅱ遺伝子単独欠損の効果によるものと結論した．

われわれは，このマウスの症状がヒト関節リウマチ（RA）でみられる関節炎と類似していることに気がついた．RAは慢性炎症性疾患で，人口の約0.5～1.0％が罹患する疾患である[6)7)]．RAでは，関節を覆う滑膜細胞が過増殖し，pannusとよばれる肉芽様の炎症組織を形成する．炎症が進むと，軟骨，骨実質が破壊され，関節部の可動性が低下するとともに，炎症部に強い痛みをもたらす．この関節炎の原因については不明の点が多いが，T細胞やB細胞，炎症性サイトカインなどが関与する自己免疫疾患と考えられている．

DNase Ⅱ変異マウスの関節部の組織切片を作製し，ヘマトキシリン—エオジン（HE）染色を行った（図B）．変異マウスでは滑膜細胞が過増殖し，さらにリンパ球，マクロファージ，好中球などの免疫細胞も浸潤しており，これらが関節腔を埋め尽くしていた．このpannusによって骨関節は浸食され，軟骨は消失していた．骨実質は激しく破壊されて骨髄と骨外部が貫通している部位も認められた．これらの炎症像はヒトのRAの組織像によく類似していた．

ところで，RAでは関節の炎症部位で，炎症性サイトカインが大量に産生されていることが報告されている．そこで，DNase Ⅱ変異マウスの関節組織における炎症性サイトカインの遺伝子発現をリアルタイムPCRによって定量した．すると変異マウスでは，正常マウスに比べ，TNF-$\alpha$が10倍，IL-1$\beta$が20倍，IL-6が100倍にも大きく発現上昇しており，このマウスでみられる関節炎がヒトRAに類似していることをさらに支持した．

## DNAの蓄積によるTNF-αの産生がDNaseⅡ変異マウスでの関節炎の原因だった

次に，なぜDNase Ⅱ遺伝子の欠損で関節炎が起こるのか，その原因を探っていった．まず，脾臓や骨髄などの造血器官において，DNAを蓄積したマクロファージが多数観察され，赤血球前駆細胞由来のDNAを蓄積していると考えられた．そして，特に骨髄において，TNF-$\alpha$遺伝子の発現が，DNase Ⅱ変異マウスでは，正常マウスに比べ，2～4倍上昇していることを見出した．血清中に含まれるTNF-$\alpha$は，正常マウスではほとんど認められないのに対し，変異マウスでは，四肢の腫脹が観察されはじめる2カ月齢以前から約100 pg/mlに達する高濃度のTNF-$\alpha$が検出された．ついで，免疫染色の結果，DNAを蓄積したマクロファージがIFN-$\beta$ばかりでなく，TNF-$\alpha$も産生していると結論した．

TNF-$\alpha$はRA発症において重要な役割を果たしている代表的な炎症性サイトカインであり，TNF-$\alpha$トランスジェニックマウスが関節炎を発症する[8)]ことから，RA発症のトリガーとも考えられている．DNase Ⅱ変異マウスでも，TNF-$\alpha$が関節炎の原因であることを確認するため，TNF-$\alpha$に対する中和抗体を，まだ腫脹が観察されない1カ月齢の時点から変異マウスに投与し，疾患の発症に与える影響を調べた．PBS投与変異マウスでは2カ月から3.5カ月の間に四肢の腫脹が観察されはじめたが，中和抗体を投与した変異マウスでは明確な腫脹は観察されなかった．また，炎症の程度を反映する，血清中のMMP-3（matrix metalloprotease-3）の値はPBS投与群ではコントロールマウスの4倍に上昇しているのに対し，抗体投与群では上昇はみられなかった．すなわち，抗TNF-$\alpha$中和抗体の投与により，このマウスでの関節炎発症は抑制された．

以上より，DNase Ⅱ変異マウスで関節炎が発症する機構は次のように考えられる．赤血球前駆細胞由来のDNAが分解されずに脾臓や骨髄のマクロファージに蓄積した結果，マクロファージは活性化されてTNF-$\alpha$を産生する．TNF-$\alpha$は体内を循環し，炎症性サイトカインに対して感受性が高いと考えられている関節の滑膜細胞を刺激し，この細胞の増殖を促す．さらにTNF-$\alpha$は，滑膜細胞に炎症性サイトカインやケモカインの産生を誘導し，炎症は増悪化し，骨破壊に至る重篤な関節炎を導く．

TNF-$\alpha$以外のサイトカインが発症に何らかの役割を果たしている可能性については，今後詳細に解析を続けていきたい．

## DNaseⅡ欠損マウスはヒト関節リウマチの理解に貢献するか

今回の結果より，成体においても，DNAの分解が

生体の恒常性の維持に重要な役割を果たしていることを明らかにできた．この結果は，われわれが提唱してきた，「DNAは遺伝情報を担う重要な分子である一方，分解されるべき局面で分解されないと生体に害を及ぼす」という考えに，さらに1つの証拠を加えるものである．また，根本原因がいまだ明らかでない関節炎において，DNAの分解異常という思いもよらない現象が，発症の原因となりうる可能性を示した．加えて，これまでRAの発症をトリガーする細胞としてはそれほど想定されていなかったマクロファージも，異常に活性化されれば，RA発症の原因となることが示唆された．ヒトの関節リウマチは，多様な原因や機構により発症する，ヘテロな疾患の集合体と考えられている．RA患者において，DNAの分解異常やマクロファージの活性化が，発症を規定する要因の1つとなっているかどうか，今後慎重に検討を進めたい．今回樹立したDNase II欠損マウスは，多くの点でヒトのRAと類似した特徴をもつ，RAのよい動物モデルとして，発症機構の解明や治療法の開発に貢献するであろう．

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### ● 筆頭著者プロフィール ●

**川根公樹**：2003年大阪大学大学院医学系研究科博士課程修了．1997年に同修士課程に入学して以来，長田重一教授のご指導のもと，DNA分解をテーマに研究を行い，現在に至る．一貫して，1つの興味深い現象（DNA分解）を対象に研究を行えてきたことに感謝し，幸せに思っている．

Review

Mesenchymal stromal cells

# Nurse-like cells reside in the synovial tissue and bone marrow in rheumatoid arthritis

Takahiro Ochi[1], Hideki Yoshikawa[2], Tomoko Toyosaki-Maeda[3] and Peter E Lipsky[4]

[1]Sagamihara National Hospital, Sagamihara, Kanagawa, Japan
[2]Department of Orthopaedic Surgery, Osaka University Medical School, Suita, Osaka, Japan
[3]Department of Immunology, Shionogi Research Laboratories, Shionogi & Co. Ltd, Osaka, Japan
[4]National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD 20892, USA

Corresponding author: Takahiro Ochi, t-ochi@sagamihara-hosp.gr.jp

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## Abstract

A major question concerning the immunopathology of rheumatoid arthritis is why the disease is localized to particular joints. A possible explanation could be the presence within the synovium of cells that foster inflammation or easy accessibility of the synovium to migratory disease enhancing cells. Within both the bone marrow and the synovium, fibroblastic stromal cells play an important role in supporting the differentiation and survival of normal cells, and also contribute to the pathologic processes. Among fibroblastic stromal cells in synovial tissue and bone marrow, nurse-like cells are a unique population having the specific capacity to promote pseudoemperipolesis (adhesion and holding beneath) of lymphocytes, and also the ability to promote the growth and function of some populations of lymphocytes and monocytes. Nurse-like cells could therefore contribute to the immunopathogenesis of rheumatoid arthritis, and may contribute to the localization of inflammation within specific joints. The present review considers the evidence that supports these possibilities.

## Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by immunologically enhanced inflammation and damage to articular structures [1,2]. Rheumatoid synovium is a site of intense inflammation, with active involvement by various populations of infiltrating lymphocytes, myeloid cells, and resident synovial fibroblasts or synoviocytes [1]. One question that has not been addressed is why RA preferentially affects certain joints. Although the explanation for the localization of rheumatoid inflammation to particular joints is not clear, one possibility relates to the presence within the synovium of resident cells that can promote inflammation. In addition, cells that can be induced to migrate from adjacent bone marrow structures may contribute to the local facilitation and propagation of inflammation and bone damage. The present review will focus on one such population, the nurse-like cells (NLCs) that populate the rheumatoid synovium and bone marrow.

## Fibroblastic stromal cells in bone marrow and synovial tissue

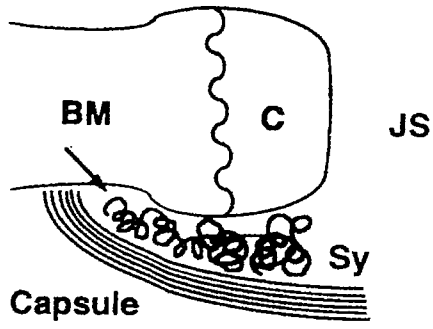
Initially, to examine the relationship between the epiphyseal bone marrow and synovial tissue, we employed the animal model of collagen-induced arthritis [3]. Fibroblastic stromal cells (FSCs) in the bone marrow of Lewis rats were labeled with a fluorescent probe or $^{3}$HTdr and were examined for their migration at the onset of arthritis [4]. Accompanying the induction of polyarthritis, a large number of labeled FSCs in bone marrow were found to migrate into the joint cavity through canals observed in the bare zone of the joint (Figure 1), and then to proliferate in the synovial tissue. This observation suggested the hypothesis that pathophysiological cells of RA could be produced in bone marrow, from which some of these cells could migrate into the joint space and potentially play roles in inflammation or tissue damage in and around articular structures. Based on these findings, we have studied FSCs of RA patients, comparing the characteristics of FSCs from bone marrow and FSCs from synovial tissue [5-7].

## Nurse-like cells found in bone marrow and synovial tissue

Among the FSCs derived from the bone marrow and synovium of RA patients, a population of NLCs was identified by the capacity to carry out pseudoemperipolesis. The

BST-1 = bone marrow fibroblastic stromal cell antigen 1; FSC = fibroblastic stromal cell; GM-CSF = granulocyte/macrophage colony-stimulating factor; HLA = human major histocompatibility antigen; IFN = interferon; IL = interleukin; mAb = monoclonal antibody; NLC = nurse-like cell; RA = rheumatoid arthritis; RANKL = receptor activator of NF-κB ligand; TNF = tumor necrosis factor; TRAP = tartarate-resistant acid phosphatase.

**Figure 1**




Migration of fibroblastic stromal cells from epiphyseal bone marrow (BM) into the joint space (JS) forming synovial (Sy) tissue in collagen-induced arthritis. C, cartilage.

function of the NLCs was reminiscent of thymic nurse cells [8,9], which have the capacity to interact with populations of thymic cells and gather them beneath their cell bodies in a process known as pseudoemperipolesis (adhesion and holding beneath). *In vivo*, such thymic nurse cells were thought to support the development and expansion of thymocytes and to also play a role in positive/negative selection of T cells in mouse and rat thymus. A very similar capacity to interact and support the maturation of some population of lymphocytes and monocytes was noted for FSCs of bone marrow [5,7] and for FSCs of synovial tissue [6,7] of RA patients, suggesting that the NLC function of FSCs could contribute to the pathophysiology of RA [7].

We established RA-NLC clones with the ability to promote pseudoemperipolesis from bone marrow [5] and synovial tissue [6] of RA patients. These RA-NLC clones were determined to be of mesenchymal origin, given that they expressed vimentin but not cytokeratin. They did not exhibit desmosomes or classical junctional complexes, both of which are characteristic features of epithelial cells. Elongated and branching mitochondria were present in the cytoplasm of the clones, and caveolae, which are unique to cells of mesenchymal origin, were present on the surface [5,6].

NLCs have a number of unique functional activities that could contribute to rheumatoid inflammation. Among these activities are their ability to promote antibody production by B cells, the capacity to protect lymphocytes from apoptosis, the ability to secrete large amounts of cytokines and chemokines that could promote the accumulation and activation of lymphocytes and monocytes, and their unique capacity to promote the differentiation of osteoclasts from myeloid precursors in a receptor activator of NF-κB/receptor activator of NF-κB ligand (RANKL)-independent manner [10].

Multipotent mesenchymal stem cells from bone marrow were also found to exist in the synovial membrane [11-14]. Those cells were shown to have multipotency to develop into various cells such as cartilage, bone, fat, and muscle. Although it is currently unknown whether these cells can differentiate into NLCs, RA-NLCs are a more differentiated population. Multipotential mesenchymal stem cells from the synovial fluid and bone marrow of patients with inflammatory and degenerative arthritis were reported to be negative for CD45 and to be positive for D7-FIB, CD13, CD105, CD55, and CD10 [13]; these mesenchymal stem cells therefore have a very different phenotype from that of RA-NLCs mentioned in the following.

## Surface phenotype of rheumatoid arthritis nurse-like cells

RA-NLC clones from bone marrow and synovial tissue [5-7] expressed CD29, CD44, CD49c, CD54, CD106, and HLA-A, HLA-B, and HLA-C (class I major histocompatibility complex), but did not express CD1a, CD18 (LFA-1), CD35, CD40, CD154, or CD56. RA-NLCs constitutively expressed CD106 after long-term culture in the absence of cytokine stimulation. Constitutive expression of CD106 appears to be a characteristic appearance of nurse cell lines, permitting them to be distinguished from fibroblasts [7]. Human dermal fibroblast also expressed CD29, CD49c, CD54, and class I major histocompatibility complex, whereas constitutive expression of CD106 was minimal. IFNγ (100 U/ml) stimulation of RA-NLCs induced expression of CD40 and HLA-DR (class II major histocompatibility complex), but not expression of CD35 or CD154. The surface phenotype of RA-NLCs was therefore similar to that of FSCs derived from synovial tissue and bone marrow cells from non-RA controls. Namely, the phenotype of NLCs derived from osteoarthritis patients and human skin nurse cells was similar to that of RA-NLCs. Enhanced expression of CD106 and CD157 by IFNγ (mentioned below) was the characteristic observation in RA-NLCs and was different from human dermal fibroblasts [7].

Expression of CD106 by RA-NLCs was modestly enhanced by culture with normal peripheral B cells, and was markedly enhanced by IFNγ. In contrast, expression of CD106 by human dermal fibroblasts was much less marked after stimulation with IFNγ or by culture with peripheral B cells. One of the features of NLCs is their capacity to promote the survival of B lymphocytes [5-7]. Such B-cell survival was reduced by a blocking anti-CD106 mAb to the same level as B cells cultured in medium alone.

One notable product of NLCs is human bone marrow fibroblastic stromal cell antigen 1 (BST-1). This product was originally cloned from a human bone marrow FSC cell line by surveying for any unknown factors [15], supporting the FSC-dependent growth of the murine pre-B-cell line DW34. A new growth factor was identified, having the ability to enhance DW34 cell growth, and it was designated BST-1 [16]. Human BST-1 is expressed in various tissues and cell

lines, such as umbilical vein endothelial cells, myeloid cells, as well as FSCs of bone marrow and also synovial cells in RA, but is not expressed in lymphoid cell lines. Notably, serum levels of BST-1 were higher (30-fold to 50-fold) in 7% of RA patients than in non-RA samples [17]. Human BST-1 was later designed as CD157, and the human *Bst-1* gene was assigned to chromosome 4q15, regulating humoral immune responses *in vivo* [18]. Expression of CD157 (BST-1) was detected on all RA-NLCs, as well as on human dermal fibroblasts. Expression of CD157 by RA-NLCs, but not by dermal fibroblasts, was enhanced by IFNγ. This enhancement was much more marked with bone marrow-derived RA-NLCs compared with synovium-derived RA-NLCs. It should be noted that expression of CD106 and CD157 mRNA was found in all RA-NLC clones. Soluble CD157 together with RA-NLCs further increased the survival of B cells, which was reduced by a blocking anti-CD157 polyclonal antibody [7].

## Cytokine production by nurse-like cells of RA patients

RA-NLCs produced numerous cytokines [5-7]. RA-NLCs from both bone marrow and synovial tissue produced detectable levels of IL-6, IL-8, and granulocyte/macrophage colony-stimulating factor (GM-CSF), and the production of IL-6 and IL-8 was quite robust. RA-NLCs from bone marrow but not synovial tissue produced IL-7, whereas RA-NLCs from synovial tissue produced granulocyte colony-stimulating factor and a greater amount of IL-6. Regulation of the production of cytokines was examined by co-culture of RA-NLCs from synovial tissue in direct contact with B cells. Secretion of IL-6, IL-8, granulocyte colony-stimulating factor, and GM-CSF was markedly increased by co-culture with B cells. IL-1β and TNF were only detected in the culture supernatants after co-culture with B cells. The effect of co-culture with B lymphocytes on the secretion of cytokines and immunoglobulin production by the B cells were examined under various culture conditions [5-7] (Table 1). After co-culture with B cells, the levels of IL-6, IL-8, granulocyte colony-stimulating factor, GM-CSF, and the levels of IgM were increased, and IL-1β and TNF were detected. Direct contact with the B-cell clone was required for RA-NLCs to produce IL-1β and TNF and higher levels of the other cytokines.

## Inhibition of spontaneous apoptosis of lymphocytes and the effect of adhesion molecules

RA-NLCs were found to promote lymphocyte viability. Although peripheral blood B cells cultured in medium alone rapidly died, culture of B cells with RA-NLCs markedly increased the B-cell viability. The loss of viability of B cells cultured alone related to the induction of apoptosis, whereas co-culture of B cells with RA-NLCs substantially blocked their apoptosis. The mechanism of the prevention of apoptosis of B cells involved the contact-dependent upregulation of Bcl-$x_L$ by RA-NLCs [19].

The regulation of pseudoemperipolesis (adhesion and holding beneath) by RA-NLCs was examined using MC/car cells and a cloned RA-NLC line from synovial tissue [20]. Pretreatment with anti-CD29 (integrin $\beta_1$ chain) or anti-CD49d (integrin $\alpha_4$ chain) reduced adhesion by MC/car cells by approximately 50%. This result indicated that integrin $\alpha_4\beta_1$ (very late antigen 4) on MC/car cells was involved, at least in part, in the cells' ability to participate in pseudoemperipolesis with RA-NLCs, although such interactions were not involved in IL-6 and IL-8 production by RA-NLCs. Pretreatment of MC/car cells with the Rho-specific inhibitor C3 transferase significantly inhibited the migration of MC/car cells underneath RA-NLCs in a concentration-dependent manner, whereas the same treatment did not inhibit the adhesion of the MC/car cells to RA-NLCs. In addition, RA-NLCs produced comparable levels of IL-6 and IL-8 when co-cultured with C3-treated transmigration-defective MC/car cells. The processes of pseudoemperipolesis, adhesion and holding beneath were therefore thought to be independent events [20]. Moreover, very late antigen 4 ($\alpha_4\beta_1$)-independent lymphocyte adhesion and not holding beneath induced the enhanced proinflammatory cytokine production by the RA-NLCs [20].

Regarding NLCs, another group reported that CD14(+) monocytes could differentiate into NLCs and support the viability of chronic lymphocytic leukemia B cells [21-23], and also support the viability of primary B cells in RA [24,25]. These effects were dependent on interactions between RA-NLC-expressed CD106 and B-cell-expressed very late antigen 4 [24], which were quite similar to the interactions between RA-NLCs and B cells we had previously reported [7]. Although the other group's NLCs were identified to be derived from CD14 myelomonocytic cells [22,23,25] we have not yet clarified the stem cell of our RA-NLCs, but it clearly appears to be of mesenchymal origin [5,6].

## RANKL-independent differentiation of osteoclast-like cells supported by RA nurse-like cells

RA-NLCs also promoted a specific pathway of the differentiation of CD14(+) monocytes. After 3–4 weeks of co-culture, CD14(+) monocytes differentiated into tartarate-resistant acid phosphatase (TRAP)(+) mononuclear cells with abundant cytoplasm and an off-center nucleus without the involvement of RANKL. It was noted that RA-NLCs supported such differentiation of peripheral blood CD14(+) monocytes not only from RA patients, but also from normal control subjects [10]. The second step of differentiation from such TRAP(+) mononuclear cells into multinucleated bone-resorbing giant cells (osteoclast-like cells) could also be induced without RANKL in the presence of IL-3, IL-5, IL-7, or GM-CSF, and was inhibited by mAb to each cytokine [10]. Differentiation of these TRAP(+) mononuclear cells into multinucleated bone-resorbing giant cells could also be promoted by macrophage colony-stimulating factor and RANKL [26].

**Table 1**

**Effects of co-culture on production of cytokines from rheumatoid arthritis nurse-like cells (RA-NLCs)**

| | Cytokines in cell culture supernatant (pg/ml)[a] | | | | | | | | | IgM (μg/ml)[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | IL-1α | IL-1β | IL-6 | IL-7 | IL-8 | G-CSF | GM-CSF | TNFα | TNFβ | Experiment 2 | Experiment 3 |
| Cytokine production from RA-NLCs derived from synovium and immunoglobulin from B cells[b] [6] | | | | | | | | | | | |
| RA-SNCs | <5.0 | <10.0 | 2,200 | | 4,300 | 460 | 40 | <5.0 | <5.0 | <1.5 | <1.5 |
| B cells | <5.0 | <10.0 | <10.0 | | <10.0 | <10.0 | <2.5 | <5.0 | <5.0 | 1.8 | 2.7 |
| B cells + RA-SNCs (separated)[c] | <5.0 | <10.0 | 1,800 | | 3,900 | 510 | 30 | <5.0 | <5.0 | <1.5 | <1.5 |
| B cells + RA-SNCs | <5.0 | 153 | 15,900 | | 34,500 | 2,400 | 740 | 690 | <5.0 | 5.6 | 8.6 |
| Cytokine production from RA-NLCs derived from bone marrow cells[d] [5] | | | | | | | | | | | |
| RA-BMNC-1 cell line | – | – | 38,250 | – | 1,480 | – | 150 | – | | | |
| + MC/car cell line | – | 320 | 89,015 | – | 33,510 | 755 | 915 | 275 | | | |
| + Molt-17 cell line | – | 235 | 78,750 | – | 10,615 | 540 | 355 | 255 | | | |

RA-BMNCs, cytokine production from RA-NLCs derived from bone marrow cells; RA-SNCs, cytokine production from RA-NLCs derived from synovium; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; –, not detectable. [a]The amount of each cytokine and IgM in the culture supernatant was measured with an enzyme-linked immunosorbent assay kit. [b]B-cell clones ($1 \times 10^5$) and RA-SNC3 ($5 \times 10^4$) were cultured under the indicated conditions for 3 days in 24-well plates. [c]B-cell clones were cultured in a Millicell culture insert. [d]RA-BMNC cells ($3 \times 10^4$ cells/well) were inoculated and cultured overnight, and $1 \times 10^6$ cells MC/car cells or Molt-17 cells were added to the culture. After 5 days of incubation, the culture supernatants were collected and the amount of each cytokine in the culture supernatant was measured with an enzyme-linked immunosorbent assay kit.

Expression of MMP-2, MMP-9, and MMP-12 was increased in both TRAP(+) mononuclear and multinucleated cells after differentiation by culture with RA-NLCs, and these cells could induce cartilage degeneration *in vitro* by a mechanism that was completely blocked by inhibitors of MMP-2 and MMP-9. Although MMP-2 expression was significantly increased in TRAP(+) mononuclear cells, expression of MMP-9 and MMP12 was also higher in TRAP(+) multinucleated cells [27]. Of note, both TRAP(+) mononuclear and multinucleated cells differentiated by culture with RA-NLCs specifically expressed MMP-12 [27], whereas multinucleated cells expressing MMP-12 were clearly found near the bone erosions (S Yamane, M Maeda-Tanimura, Y Shimaoka, M Yukioka, T Toyosaki-Maeda, S Ishida, N Yamane, Y Tsuruta, T Itoh, N Fukui, *et al.*, unpublished observation). RA-NLCs were therefore found to promote the differentiation of CD14(+) monocytes in a characteristic two-step differentiation process into multinucleated osteoclast-like cells with the capacity to degrade bone and cartilage.

Although TNF [28], IL-1 [29], macrophage colony-stimulating factor, and RANKL [30] are very important factors for developing osteoclasts, the RANKL-independent two-step differentiation of CD14(+) monocyte supported by RA-NLCs [10,26] may be an alternative pathway to develop multinucleated osteoclast-like cells specifically in RA. Beside the destruction of bone tissue by osteoclasts or osteoclast-like cells, we could confirm that FSCs from RA patients inoculated *in vivo* showed aggressive behavior, invading cartilage as reported previously [31-33], although we have not yet confirmed that pure RA-NLC lines have such function.

## Comparison of the properties of RA nurse-like cells and fibroblast-like synoviocytes

A considerable amount of work has characterized another population of cells found in the rheumatoid synovium, namely fibroblast-like synoviocytes. The cells are thought to play a role in rheumatoid pathogenesis, especially because of their capacity to contribute to tissue damage [31-33]. RA-NLCs, however, have a number of specific attributes that suggest they may play a unique role in RA pathogenesis (Table 2).

## Mechanisms of progressive proliferation of fibroblastic stromal cells specifically found in joint

To explain the remarkable proliferation of synovial tissue in the RA patient, various mechanisms have been reported such as the involvement of protooncogenes [34], inflammatory cytokines [35], and perturbations of Fas-mediated apoptosis [36]. As a mechanism specifically found in the synovial space but not in the bone marrow, we found that the interference with Fas-mediated apoptosis could upregulate specifically the growth of synovial FSCs [37,38]. In this regard, soluble Fas ligand was found to inhibit competitively the Fas–Fas ligand-mediated apoptosis [37] of FSCs bearing Fas. The levels of human soluble Fas ligand in synovial fluid from RA patients were found to be significantly higher than those from osteoarthritis patients.

**Table 2**

**Comparison of the properties of rheumatoid arthritis nurse-like cells and fibroblast-like synoviocytes**

| Property | Rheumatoid arthritis nurse-like cells | Fibroblast-like synoviocytes |
|---|---|---|
| Pseudoemperipolesis | + | – |
| Constitutive expression of CD106 | + | – |
| Enhanced expression of CD106 and CD157 by IFNγ | + | – |
| Promote B-cell differentiation | + | – |
| Promote differentiation of osteoclast-like cells from CD14(+) monocytes | + | – |
| Inhibit lymphocyte apoptosis | + | – |

In contrast, soluble Fas ligand was not detected in the peripheral blood, and also not in bone marrow blood in RA patients [38]. This mechanism, therefore, could at least partially upregulate the FSC growth in synovial tissue, but not in bone marrow.

## Conclusion

A specific population of FSCs, RA-NLCs reside in both the bone marrow and synovium of RA patients and have the functional capacity to interact with lymphocyte and monocyte populations, inducing cellular differentiation and biologic activities that mimic pathophysiologic features of rheumatoid inflammation. These findings suggest that RA-NLCs may play an essential role in the development of local immune and inflammatory responses in the synovium and the bone marrow. RA-NLCs could therefore be central elements in the pathologic events in RA and might be appropriate targets for therapeutic intervention in RA.

## Competing interests

The authors declare that they have no competing interests.

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第7回日本骨粗鬆症学会奨励賞受賞演題

# アレンドロネート投与後の骨量改善に伴う動脈硬化進展に及ぼす影響について

山田真介[1)]・稲葉雅章[1)]・後藤仁志[1)]・田中一匡[1)]
酒井真礼[2)]・白川久美[1)]・今西康雄[1)]・西沢良記[1)]

## はじめに

以前より，骨粗鬆症の進展と動脈硬化進展の関連が示唆されている。たとえば，脳血管障害による片側不全麻痺患者では，身体活動性の低下した麻痺側において，健側と比較し有意な骨量の減少と動脈硬化の進展を認めており，両者の間には有意な負の相関を認める[1)]。同様に，関節リウマチ患者(RA患者)では，健常人と比較して骨量が低下し[2)]，動脈壁肥厚度が進展している[3)]。つまりRA患者においても，骨量の減少と動脈硬化進展の間には何らかの関連が存在することが示唆される。また，一般女性と比較して女性RA患者では，心血管病変による死亡リスクが高いという報告がある[4)]。つまり，RA患者では一般の人々と比較し，動脈硬化の管理が非常に重要であることが示唆される。

## 1 RA合併症検査

当科ではRAに伴う内科合併症を検討する目的で，通院中のRA患者に対し動脈硬化の進展度と骨量を定期的に検査している。動脈硬化の評価については，動脈壁硬化度の指標となる上腕-足首間の脈波伝播速度(ba PWV)を測定し，骨量については体幹の骨量として腰椎骨密度(腰椎BMD)を，四肢の骨量として大腿骨・踵骨の骨密度(大腿骨BMD)，踵骨OSIを測定している。また，骨代謝の状態を検討するために，骨吸収の指標として尿中NTX/Creを，骨形成の指標としてBAPを測定している。

## 2 健常人とRA患者の骨量と動脈壁硬化度の比較

現在，当科通院加療中の閉経後RA患者47名と，年齢・BMI・収縮期血圧をマッチさせた，一般の閉経後女性47名の骨量と動脈壁硬化について比較検討してみると，RA患者では有意に腰椎BMDおよび踵骨OSIが低下し，ba PWVが上昇していることがわかる(**表1**)。さらに，腰椎BMDも踵骨OSIもba PWVとの間に有意な負の相関を認めている(**表2**)。つまり，RA患者は身体活動性の低下などを危険因子とし早期から骨量減少をきたしており，その程度に伴い動脈硬化が進展していると示唆される。

## 3 目的と方法

RA患者の骨量を改善することにより，動脈壁硬化の進展を予防する可能性も示唆されることから，骨吸収抑制剤であるアレンドロネート

**Influence on Atherosclerosis with the Improvement of Bone Mineral Density after Medication for Osteoporosis in Arthritis Rheumatism Patient**

Shinsuke Yamada: Department of Metabolism, Endocrinology, and Molecular Medicine Osaka City University Gradute School of Medecine, et al.

**Key words :** Osteoporosis, Atherosclerosis, Alendronate , Arthritis rheumatism

[1)]大阪市立大学大学院医学研究科代謝内分泌病態内科学，[2)]阪和第二泉北病院

表1　健常人とRA患者の骨量と動脈硬化進展の比較

| | コントロール | RA | $p$ |
|---|---|---|---|
| 人数(閉経後女性) | 47 | 47 | -- |
| 年齢(歳) | 60.0 ± 5.9 | 60.0 ± 9.7 | 0.9898 |
| BMI(kg/m$^2$) | 22.1 ± 3.3 | 21.3 ± 3.3 | 0.2628 |
| 収縮期血圧(mmHg) | 125.0 ± 19.8 | 128.9 ± 20.3 | 0.3551 |
| Total cholesterol(mg/dL) | 224.9 ± 29.6 | 204.3 ± 32.9 | 0.0024 |
| CRP(mg/dL) | -- | 1.3 ± 1.9 | -- |
| U-NTX/Cre(nmol BCE/mmol・Cr) | -- | 83.9 ± 37.0 | -- |
| 腰椎BMD(g/cm$^2$) | 0.874 ± 0.100 | 0.820 ± 0.154 | 0.0498 |
| 踵骨OSI(×10$^6$) | 2.495 ± 0.269 | 2.196 ± 0.266 | <0.0001 |
| ba PWV (cm/s) | 1301.1 ± 214.6 | 1573.0 ± 381.9 | <0.0001 |

表2　ba PWVと各パラメータの相関

| | ba PWV(cm/s) | | | |
|---|---|---|---|---|
| | コントロール | | RA | |
| | $r$ | $p$ | $r$ | $p$ |
| 年齢(歳) | 0.284 | 0.0532[§] | 0.627 | <0.0001[‡] |
| BMI(kg/m$^2$) | -- | 0.5140 | -- | 0.7466 |
| 収縮期血圧(mmHg) | 0.595 | <0.0001[‡] | 0.625 | <0.0001[‡] |
| Total Chol(mg/dL) | -- | 0.8802 | -- | 0.2529 |
| CRP(mg/dL) | -- | -- | -- | 0.5697 |
| 腰椎BMD(g/cm$^2$) | -- | 0.1461 | −0.299 | 0.0460[†] |
| 踵骨OSI(×10$^6$) | -- | 0.6007 | −0.309 | 0.0437[†] |

[‡]$p<0.0001$, [†]$p<0.05$, [§]$p<0.1$

(5mg/日)を投与し，動脈壁硬化の進展を予防しうるか否かにつき検討した。対象はRA患者16名，アレンドロネート非投与群，アレンドロネート投与群おのおの8名ずつ，すべて女性とした。アレンドロネート投与前および投与半年から1年後において，ba PWV，腰椎・大腿骨BMD，尿中NTX/Cre，BAPを測定した。対象の開始時の背景を表3に示す。

## 4　結　　果

観察前後での各種パラメータの結果を表4，5に示す。血清CRP値は，非投与群・投与群ともにほとんど変化を認めなかった。骨代謝の状態については，非投与群において，NTX/CreおよびBAPとも有意な上昇を認め，骨代謝が亢進していることが示唆された。一方，投与群では，NTX/Creは有意に減少しており，骨吸収が抑制されていることが示唆された。腰椎BMDについては，非投与群では変化を認めなかったが，投与群では有意な上昇を認めた。大腿骨BMDにおいても，非投与群では変化を認めなかったが，投与群では上昇傾向を認めた。つまり，投与群ではアレンドロネートが骨吸収を抑制することにより骨量が増加したと考えられた。ba PWVの変化については，統計学的に有意な結果とはならなかったが，その平均値は非投与群において約4%の増加を示し，投与群では約8%の減少を認めた。また，腰椎BMDとba PWVそれぞれの増加率は有意な負の相関($r=0.671$, $p=0.0337$)を認めた。つまり，骨量が増加すればするほど動脈壁硬化が改善することが示唆された。

表3　対象者の背景（開始時）

| | 非投与群 | 投与群 | $p$ |
|---|---|---|---|
| 閉経前/後 | 2/6 | 1/7 | — |
| 年齢(歳) | 62.6 ± 12.6 | 59.9 ± 10.0 | 0.6962 |
| BMI($kg/m^2$) | 22.5 ± 2.9 | 19.8 ± 2.7 | 0.0678 |
| 収縮期血圧(mmHg) | 127.5 ± 13.9 | 135.5 ± 17.5 | 0.3286 |
| CRP(mg/dL) | 0.450 ± 0.396 | 1.671 ± 1.598 | 0.0558 |
| 腰椎BMD($g/cm^2$) | 0.882 ± 0.207 | 0.846 ± 0.205 | 0.7560 |
| 大腿骨BMD($g/cm^2$) | 0.761 ± 0.182 | 0.707 ± 0.193 | 0.6519 |
| ba PWV(cm/s) | 1637 ± 257 | 1652 ± 538 | 0.9532 |
| NTX/Cre(nmol BCE/mmol・Cr) | 64.2 ± 23.6 | 60.2 ± 17.2 | 0.7753 |
| BAP(IU/L) | 22.8 ± 8.3 | 22.8 ± 5.1 | 0.9973 |

表4　観察前後での各種パラメータの結果（アレンドロネート非投与群）

| | 前 | 後 | $p$ |
|---|---|---|---|
| CRP(mg/dL) | 0.450 ± 0.396 | 0.471 ± 0.519 | 0.7261 |
| 腰椎BMD($g/cm^2$) | 0.882 ± 0.207 | 0.882 ± 0.181 | 0.9916 |
| 大腿骨BMD($g/cm^2$) | 0.761 ± 0.182 | 0.761 ± 0.191 | >0.9999 |
| baPWV(cm/s) | 1637 ± 257 | 1690 ± 241 | 0.5395 |
| NTX/Cre(nmol BCE/mmol・Cr) | 64.2 ± 23.6 | 90.9 ± 24.9 | 0.0009† |
| BAP(IU/L) | 22.8 ± 8.3 | 32.3 ± 9.4 | 0.0504 |

† $p<0.05$

表5　観察前後での各種パラメータの結果（アレンドロネート投与群）

| | 前 | 後 | $p$ |
|---|---|---|---|
| CRP(mg/dL) | 1.671 ± 1.598 | 1.725 ± 1.642 | 0.9231 |
| 腰椎BMD($g/cm^2$) | 0.846 ± 0.205 | 0.884 ± 0.203 | 0.0014† |
| 大腿骨BMD($g/cm^2$) | 0.707 ± 0.193 | 0.736 ± 0.168 | 0.0837§ |
| baPWV(cm/s) | 1652 ± 538 | 1479 ± 306 | 0.2729 |
| NTX/Cre(nmol BCE/mmol・Cr) | 60.2 ± 17.2 | 35.4 ± 22.8 | 0.0028† |
| BAP(IU/L) | 22.8 ± 5.1 | 22.7 ± 16.3 | 0.9833 |

† $p<0.05$, § $p<0.1$

## おわりに

アレンドロネートは，RA患者で骨吸収を有意に抑制し，骨量を有意に増加することが示された。また，骨量の改善に伴い動脈硬化の進展を予防する可能性が示唆された。

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Osteoporosis Japan

# 関節リウマチ患者では傍関節性骨粗鬆症の進展が末梢の動脈硬化進展に関与する

田中一匡　稲葉雅章　櫻井真由美
山田真介　酒井真礼　後藤仁志
西沢良記

## はじめに

関節リウマチ(RA)患者において動脈硬化が進行していることが知られている[1~4]。

われわれはRA患者の超音波Bモード法で測定した総頸動脈内膜中膜肥厚度(CCA-IMT)が有意に増加していることを見出し，RAが動脈壁肥厚度(thickening)の独立した危険因子であることを報告した[5]。さらに，RA炎症と骨吸収亢進がその重要な関与因子であることを示した[6]。今回，これら因子とRA患者の動脈壁の硬化度

表1　関節リウマチ患者群と対照群における臨床パラメータ

| | 対照群 | 関節リウマチ患者群 | p |
|---|---|---|---|
| 人数 | 49 | 47 | |
| 年齢(歳) | 56.7 ± 7.4 | 59.6 ± 14.1 | 0.198 |
| Body mass index (kg/m$^2$) | 20.4 ± 2.2 | 21.1 ± 2.4 | 0.240 |
| 喫煙 / 非喫煙 | 4/45 | 2/45 | 0.240 |
| 総コレステロール(mg/dL) | 209.5 ± 19.0 | 198.8 ± 34.2 | 0.058 |
| LDLコレステロール(mg/dL) | 116.5 ± 31.5 | 110.6 ± 24.9 | 0.346 |
| 収縮期血圧(mmHg) | 129.3 ± 17.3 | 131.9 ± 21.4 | 0.505 |
| CRP(mg/dL) | ND | 1.0 (0.1 ~ 8.0) | — |
| RF(IU/mL) | ND | 151.1 (9 ~ 1270) | — |
| ESR(mm/hr) | ND | 49 (8 ~ 110) | — |
| 血小板数(× 10$^4$/μL) | ND | 26.3 (17.2 ~ 56.1) | — |
| DPD/Cre(nmol/mmol Cr) | ND | 7.9 (4.8 ~ 21.2) | — |
| NTX/Cre(nmol BCE/mmol Cr) | ND | 69.5 (29.3 ~ 153.9) | — |

表中の数字は平均値±標準偏差を示す。
ND：測定値なし，LDL：low density lipoprotein，CRP：C-reactive protein，ESR：erythrocyte sedimentation rate，DPD：deoxypyridinoline，NTX/Cre：N-terminal telopeptide/creatinine ratio

**Involvement of Paraarticular Trabecular Bone Loss at the Ultradistal Radius in Increased Arterial Stiffening in Postmenopausal RA Patients**

Kazumasa Tanaka: Department of Metabolism, Endocrinology, and Molecular Medicine, Osaka City University Graduate School of Medicine, *et al.*

**Key words**: Atherosclerosis, Rheumatoid arthritis, Arterial stiffening, Pulse wave velocity, Paraarticular osteoporosis

大阪市立大学大学院医学研究科代謝内分泌病態内科学

表2　関節リウマチ患者群と対照群における RA 罹患と faPWV, baPWV との関連をみた多変量解析

| 独立変数 | Log faPWV | | | Log baPWV | | |
|---|---|---|---|---|---|---|
| | モデル1 | モデル2 | モデル3 | モデル1 | モデル2 | モデル3 |
| 年齢 | 0.153 | 0.190 | 0.186 # | 0.398 * | 0.435 * | 0.439 * |
| 収縮期血圧 | 0.339 * | 0.328 $ | 0.378 * | 0.418 * | 0.423 * | 0.427 * |
| RA 罹患 | 0.316 $ | 0.294 $ | 0.256 $ | 0.264 * | 0.188 * | 0.188 # |
| 喫煙 / 非喫煙(− / +) | − 0.049 | | | 0.026 | | |
| 総コレステロール値 | | − 0.023 | | | − 0.023 | |
| Body mass index | | | − 0.131 | | | − 0.102 |
| 重相関係数 | 0.318 * | 0.301 * | 0.328 * | 0.541 * | 0.539 * | 0.552 * |

表中の数字は標準偏回帰係数($\beta$)を示す。* $p < 0.001$, $ $p < 0.01$, # $p < 0.05$

表3　RA 患者 47 例における臨床パラメータと faPWV, baPWV との単相関

| | Log faPWV | | Log baPWV | |
|---|---|---|---|---|
| | 標準偏回帰係数 | $p$ 値 | 標準偏回帰係数 | $p$ 値 |
| 年齢 | 0.192 | 0.213 | 0.520 | 0.001 * |
| 罹病期間 | 0.108 | 0.537 | 0.110 | 0.530 |
| Log 橈骨遠位端総骨密度 | − 0.078 | 0.631 | − 0.294 | 0.060 |
| Log 橈骨遠位端海面骨密度 | − 0.425 | 0.007 $ | − 0.553 | < 0.001 * |
| Log 踵骨 OSI | − 0.021 | 0.893 | − 0.357 | 0.017 # |
| Log CRP | − 0.059 | 0.711 | 0.002 | 0.988 |
| Log RF | − 0.001 | 0.997 | 0.017 | 0.630 |
| Log ESR | 0.096 | 0.588 | 0.115 | 0.519 |
| Log DPD/Cre | − 0.011 | 0.965 | 0.065 | 0.799 |
| Log NTX/Cre | − 0.076 | 0.642 | 0.046 | 0.779 |

BMD：bone mineral density, CRP：C-reactive protein, ESR：erythrocyte sedimentation rate, DPD：deoxypyridinoline, NTX：N-terminal telopeptide, Cre：Creatinine
* $p < 0.001$, $ $p < 0.01$, # $p < 0.05$

(stiffening)と末梢の骨密度の低下との関連を調べた。

## 1　対象と方法

高血圧，高脂血症など動脈硬化に影響を及ぼす疾患を有さない閉経後の47例の女性RA患者と54人の健常者につき検討した。2群間の年齢，BMI，総コレステロール値，LDLコレステロール値，収縮期血圧に有意差は認めなかった(表1)。動脈壁 stiffening を Colin 社製 form PWV/ABI(model BP-203RPE)を用いて Pulse Wave Velocity(PWV)で評価した。全身性骨粗鬆症として踵骨 osteo-sono index(OSI)を Aloka 社製 AOS-100 による超音波法により測定し算出した。RAの傍関節性骨粗鬆症の指標として橈骨遠位端の骨密度を海綿骨部と皮質骨部とに分けて Stratec 社製 XCT-960 による pQCT 法にて測定した。さらに RA 疾患活動性マーカーとして CRP，ESR，血小板，RFを，骨吸収マーカーとして尿中デオキシピリジノリンとNTXを，さらに動脈硬化の既知因子を測定し検討した。

## 2　結　　果

RA 患者での femoral-ankle(fa)PWV と brachial-ankle(ba)PWV は 1124(range 880～1429) cm/sec，1539(range 946～2501)cm/secで，健

表 4　RA 患者 47 例における faPWV, baPWV と臨床パラメータとの関連をみた多変量解析

| Independent variables | Log faPWV | | | Log baPWV | | |
|---|---|---|---|---|---|---|
| | モデル 1 | モデル 2 | モデル 3 | モデル 1 | モデル 2 | モデル 3 |
| 年齢 | 0.193 | 0.325 | 0.220 | 0.337 $^{\$}$ | 0.421 $^{\$}$ | 0.421 $^{\$}$ |
| 喫煙 / 非喫煙 (− / +) | 0.100 | 0.025 | − 0.050 | 0.105 | 0.076 | 0.121 |
| 収縮期血圧 | 0.378 $^{\#}$ | 0.386 $^{\#}$ | 0.375 $^{\#}$ | 0.452 $^{*}$ | 0.482 $^{*}$ | 0.420 $^{\$}$ |
| Log 橈骨遠位端海面骨密度 | − 0.325 $^{\#}$ | | − 0.360 $^{\$}$ | | | |
| Log 橈骨遠位端総骨密度 | − 0.006 | − 0.201 | | | | |
| Log 踵骨 OSI | | 0.157 | − 0.081 | | | |
| 重相関係数 | 0.426 $^{*}$ | 0.358 $^{\$}$ | 0.230 $^{\#}$ | 0.711 $^{*}$ | 0.655 $^{*}$ | 0.562 $^{*}$ |

表中の数字は標準偏回帰係数 ($\beta$) を示す。$^{*}p < 0.001$, $^{\$}p < 0.01$, $^{\#}p < 0.05$

常者の 982(range 739 ～ 1442)cm/sec, 1322 (range 1031 ～ 2212)cm/sec に比し，有意な上昇が認められた。RA患者群と対照群において血圧，年齢，喫煙の有無で補正した多変量解析で RA 罹患は独立した動脈壁 stiffening の危険因子であった(表2)。RA患者群において橈骨遠位端の海綿骨部の骨密度はfaPWV, baPWVと有意な負の相関を認めた(表 3)。

橈骨遠位端の海綿骨部の骨密度は RA 患者で健常者に比べて有意な低下を認め，多変量解析で橈骨海綿骨骨密度の低下がRA患者の動脈壁stiffening 進展の独立した危険因子であった(表 4)。

## 3　結論と考察

閉経後女性 RA 患者では傍関節性骨粗鬆症の進展が末梢の動脈壁stiffening進展の危険因子であることが示唆された。橈骨遠位端における海綿骨骨密度の低下は RA の炎症に特徴的である[7]。さらにRAに伴う関節炎のある関節は血清中のピリジノリンとデオキシピリジノリンの上昇に寄与していることも知られている[8]。以上のことより橈骨遠位端における海綿骨骨密度の低下と末梢の動脈壁stiffeningの進展との関連が考えられる。

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# Associations between physical activity, peripheral atherosclerosis and bone status in healthy Japanese women

Shinsuke Yamada, Masaaki Inaba*, Hitoshi Goto, Mayumi Nagata-Sakurai, Yasuro Kumeda, Yasuo Imanishi, Masanori Emoto, Eiji Ishimura, Yoshiki Nishizawa

*Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan*

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**Abstract**

The aim of this cross-sectional study was to investigate whether physical activity and bone status may affect arterial thickening and stiffening in healthy Japanese women. Healthy women ($n=149$; mean age, 54 years) were recruited from those who participated in a local health check program at the Osaka City University Hospital. Physical activity was assessed by physical functioning score of SF-36, and bone status by bone mineral density (BMD) in lumbar spine and calcaneus osteo-sono index (OSI). Arterial wall thickening assessed by intima–media thickness (IMT) in common carotid artery (CA) and femoral artery (FA), and arterial wall stiffening by peak wave velocity (PWV) in heart–carotid (hc) and heart–femoral (hf) as central segment and in heart–brachial (hb) and femoral–ankle (fa) as peripheral segment, respectively. By Spearman Rank correlation, lumbar spine BMD was correlated negatively with CA IMT ($\rho=-0.225$, $p<0.05$) and FA IMT ($\rho=-0.215$, $p<0.05$), and calcaneus OSI with FA IMT ($\rho=-0.330$, $p<0.0001$) but not CA IMT ($\rho=-0051$, $p=0.5335$). Both lumbar spine BMD and calcaneus OSI correlated negatively with PWV in all segments (all $p<0.05$). Physical functioning score correlated weakly but significantly in a negative manner with all PWV segments (all $p<0.05$) but not IMT. Multiple regression analyses revealed a significant association of calcaneus OSI ($\beta=-0.240$, $p=0.0039$) but not lumbar spine BMD ($\beta=-0.067$, $p=0.4541$) with FA IMT, although neither lumbar spine BMD nor calcaneus OSI was associated with CA IMT. Furthermore, physical functioning score was independently associated with hb and fa PWV but not hc and hf PWV, suggesting the preferential association with peripheral segment including lower extremities. Neither lumbar spine BMD nor calcaneus OSI was associated with any segment of PWV.

In conclusion, it was suggested that calcaneus OSI might be associated with arterial wall thickening preferentially in femoral artery, and that physical activity may be associated with arterial wall stiffening in peripheral segment including lower extremity but not in central segment in healthy Japanese women.



*Keywords:* Osteoporosis; Quality of life; ADL; Bone mineral density; Atherosclerosis

## 1. Introduction

Atherosclerosis and osteoporosis progress simultaneously with advancing age [1] and shares common risk factors such as smoking [2] and menopause [3]. There is an association between aortic calcification and BMD in hip or lumbar spine in post-menopausal women, therefore, suggesting the development of osteoporosis as a risk for advanced atherosclerosis after menopause [1,4].

Atherosclerosis has two key components, arterial wall thickening (atherosis) and arterial wall stiffening (sclerosis), which now can be quantified by measuring far wall intimal–medial thickness (IMT) and pulse wave velocity (PWV), respectively. The IMT of the common carotid artery (CA) and femoral artery (FA) has been established as a clinically useful index for identifying early-stage general and local atherosclerosis in lower extremities [5–11], respec-

* Corresponding author. Tel.: +81 6 6645 3806; fax: +81 6 6645 3808.
*E-mail address:* inaba-m@med.osaka-cu.ac.jp (M. Inaba).

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tively, since CA IMT is strongly correlated with the presence of coronary artery diseases [5–10] and FA IMT with local atherosclerosis [11]. PWV is also established as a marker of early-stage atherosclerosis [12]. Heart–carotid (hc) PWV and heart–femoral (hf) PWV reflect central segment of atherosclerosis and heart–brachial (hb) PWV and femoral–ankle (fa) PWV peripheral segment, respectively [13].

Physical activity is also one of the important factors affecting atherosclerosis. Weekly fitness activity is a significant profitable factor against the development of aortic calcification [1]. Conversely, arterial stiffening was increased in the paretic lower limb of hemiparetic patients [14]. Recently, the Medical Outcomes Study 36-item Short Form (SF-36), which is a self-administered questionnaire containing 36 items that, when scored, yield eight domains considering physical, cognitive, emotional and social aspects [15,16] has emerged as a valuable index to assess health-related quality of life (HRQL) [17].

Furthermore, lumbar spine (L2–4) BMD and calcaneus osteo-sono-assessment index (OSI) are measured as index of osteoporosis [18,19].

This background prompted us to examine the association of physical activity as reflected by SF-36 score and of bone status as reflected by lumbar spine BMD and calcaneus OSI with general and local arterial wall thickening as reflected by IMT and central and peripheral arteries stiffening as reflected by PWV in healthy Japanese women.

## 2. Subjects and methods

### 2.1. Subjects

Healthy Japanese women ($n = 149$) were recruited from people who participated in a local health check program at the Osaka City University Hospital from July to September in 2002 after written informed consent was obtained. The mean age was 54.1 ± 12.3 years. Pre- and post-menopausal women were 46 and 103, respectively. Exclusion criteria are the subjects who are known to be suffering from any major diseases which might affect atherosclerosis and bone metabolism, such as diabetes mellitus (all subjects were HbA1c ≦ 5.7%), cerebral vascular accident impairing seriously activity of daily life (ADL), intermittent claudication, osteoporosis and osteomalacia. The subjects who have been continuously taking medicines were also excluded from the present study.

### 2.2. IMT measurement

Ultrasonographic examination of the CA and FA was performed in the supine position by high-resolution ultrasonography with a 10 MHz in-line Sectascanner (SSD 610 CL; Aloka Co., Tokyo, Japan), as previously described [20–25]. To avoid inter-observer variability, all measurements were performed by the same examiner (H.Y.) who was unaware of subject characteristics. Briefly, CA and FA were scanned at the level of the bifurcation on both the right and left sides. IMT was measured in the far wall of the CA and FA at sites of the most advanced arterial thickening as diffuse and continuous projection with the greatest distance between the lumen–intimal interface and the media–adventitial interface but without atherosclerotic plaque, which was defined as localized lesions of thickness from 1.3 to 1.5 mm, from digitized still images of the arteries during scanning [26]. These interfaces were all manually traced on the same day to avoid possible variation during the study period and the mean value calculated as the mean of at least 3 still images obtained from the same section of the CA [20,22]. Reproducibility of the IMT measurement was acceptable as shown by coefficients of variation (CV) of 2.8 and 3.4% for CA IMT and FA IMT, respectively. These were calculated from the 40 measurements performed in 20 RA patients on two different occasions according to Bland and Altman [27] using the following formula;

$$\mathrm{CV}(\%) = \frac{100\left(\frac{\mathrm{S.D.}}{\sqrt{2}}\right)}{x}$$

where S.D. is the standard deviation of absolute differences between the two repeated measurements and $x$ is the pooled mean value.

### 2.3. PWV measurement

PWV was measured in the supine position after 5 min of bed rest using an automatic form analyzer (model BP-203RPE; Colin, Komaki, Japan). Pressure waveforms of the brachial and tibial arteries were recorded by an oscillometric method using the occlusion/sensing cuffs adapted to both arms and both ankles. Pressure waveforms of the carotid and femoral arteries were recorded using multielement tonometry sensors placed at the left carotid and the left femoral arteries. Electrocardiogram was monitored with electrodes placed to both wrists. Heart sound S1 and S2 were detected by a microphone set on the left edge of the sternum at the third intercostals space.

The waveform analyzer measures time intervals between S2 and the notch of carotid pulse wave (Thc), between S2 and the notch of brachial pulse wave (Thb), between pulse wave of the carotid and femoral arteries (Tcf), and between pulse wave waves of the femoral and tibial (ankle) arteries (Tfa). The sum of Thc and Tcf gives the time for pulse waves to travel from the heart (aortic orifice) to the femoral artery (Thf). Also, the waveform analyzer estimates the path lengths of the heart–carotid (Dhc), the heart–brachial (Dhb), the heart–femoral (Dhf) and the femoral–ankle (Dfa) segments based on the height (HT, cm) using the following formulas; $\mathrm{Dhc} = 0.2473 \times \mathrm{HT} - 18.999$; $\mathrm{Dhb} = 0.2195 \times \mathrm{HT} - 2.0734$; $\mathrm{Dhf} = 0.5643 \times \mathrm{HT} - 18.381$; $\mathrm{Dfa} = 0.2486 \times \mathrm{HT} - 30.709$. PWV was calculated for each

arterial segment as the path length divided by the corresponding time interval.

Reproducibility of the PWV measurement was evaluated by repeating measurements in 17 healthy subjects on two different occasions. The coefficients of variation were 6.0, 3.3, 4.9 and 3.3% for hc PWV, hb PWV, hf PWV and fa PWV, respectively [13].

### 2.4. Assessment of health-related quality of life (HRQL)

HRQL was assessed by means of SF-36 [15,16]. The questionnaire consists of 36 items and measures three aspects of health: functional ability, well-being and overall health. These are quantified using eight multi-item domains (physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional and mental health). The physical functioning domain assesses limitations in physical activities such as walking and climbing stairs. The role-physical and role-emotional domains measure problems with work or other daily activities as a result of physical health or emotional problems. Bodily pain assesses limitations resulting from pain; vitality measures energy and tiredness. The social functioning domain examines the effect of physical and emotional health on normal social activities, and mental health assesses happiness, nervousness and depression. The general health perceptions domain evaluates the personal opinion of one's health compared with that of one's peers, as well as the expectation of changes in health. All domains are scored on a scale from 0 to 100, with 100 representing the best possible health state. Two summary scales (physical and mental component) can also be derived [16,28].

The SF-36 has been validated for use to assess HRQL in osteoporotic patients [29,30].

### 2.5. BMD measurement at lumbar spine

BMD was measured in the lumbar spine (L2–4) in the anterior–posterior projection by dual-energy X-ray absorptiometry (DEXA; QDR-4500A, Hologic Inc., Waltham, MA), essentially as previously described [31]. The precision of the measurement of lumbar spine BMD using DEXA was less than 1.8%.

### 2.6. Quantitative ultrasound assessment of calcaneus

Quantitative ultrasound assessment of calcaneus was performed using an ultrasound system (Acoustic Osteo-Screener (AOS-100), Aloka Co. Ltd., Tokyo, Japan), as previously described [20,32]. Briefly, the AOS-100 measures both speed of sound (SOS) and an attenuation-related parameter called the transmission index (TI). These measurements yield a derived parameter, the osteo-sono-assessment index (OSI), which has been proposed to be an estimate of the elastic modulus of the calcaneus [33]. In fact, evidence has been accumulated to indicate that calcaneus OSI, a parameter of quantitative ultrasound assessment of the calcaneus, provides a useful index to estimate elastic modules of the calcaneus [33], and thus a good parameter mostly related to bone mineral density [34]. Precision of the OSI parameter was 2.2% [34].

### 2.7. Statistical analysis

Values are expressed as mean ± S.D. unless otherwise indicated. Statistical analysis was performed with the Stat View V system (Abacus Concepts, Berkeley, CA) for the Apple computer. The correlation coefficients were calculated by simple regression analysis. $p$-values of less than 0.05 were considered as statistically significant. Multiple regression analysis was performed to assess independent association with IMTs and PWVs. $p$-values of less than 0.05 were considered as statistically significant.

## 3. Results

### 3.1. Clinical variables, IMT, PWV and bone density of healthy Japanese women

Clinical characteristics of healthy Japanese women ($n = 149$) enrolled in this cross-sectional study are shown in Table 1. The means of systolic and diastolic blood pressure, HDL and LDL cholesterol, and triglycerdides were within normal range. The means of CA and FA IMT were to the value 0.597 ± 0.148 and 0.838 ± 0.385 mm, respectively, and the mean values were 861.6 ± 288.3 cm/s for hc PWV, 867.4 ± 192.1 cm/s for hf PWV, 555.9 ± 70.8 cm/s for

Table 1
Clinical characteristics of 149 women in this study

| | |
|---|---|
| Age (year) | 54.1 ± 12.3 |
| BMI (kg/m$^2$) | 21.8 ± 3.1 |
| Non/menopause | 46/103 |
| Systolic BP (mmHg) | 121.0 ± 20.1 |
| Diastolic BP (mmHg) | 67.5 ± 10.0 |
| Smoking index | 36.1 ± 142.9 |
| Smokers/non-smokers | 17/132 |
| HDL cholesterol (mg/dl) | 66.6 ± 16.0 |
| LDL cholesterol (mg/dl) | 133.6 ± 31.7 |
| Triglyceride (mg/dl) | 90.1 ± 42.5 |
| CA IMT (mm) | 0.597 ± 0.148 |
| FA IMT (mm) | 0.838 ± 0.385 |
| hc PWV (cm/s) | 861.6 ± 288.3 |
| hf PWV (cm/s) | 867.4 ± 192.1 |
| hb PWV (cm/s) | 555.9 ± 70.8 |
| fa PWV (cm/s) | 940.3 ± 157.4 |
| Lumbar spine BMD (g/cm$^2$) | 0.876 ± 0.146 |
| Calcaneus OSI ($\times 10^6$) | 2.54 ± 0.28 |

Data are expressed as mean ± S.D. BMI: body mass index, BP: blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein, CA IMT: common carotid artery–intima–media thickness, FA: femoral artery, hc PWV: heart–carotid pulse wave velocity, hf: heart–femoral, hb: heart–brachial, fa: femoral–ankle, BMD: bone mineral density, OSI: osteo-sono-assessment index.

Table 2
Unadjusted domain scores of the eight subscales and adjusted domain scores of two summary scales of the SF-36 scores in 149 healthy Japanese women

| | |
|---|---|
| Scores in SF-36 | |
| Physical functioning | 89.9 ± 9.4 |
| Role-physical | 90.0 ± 23.1 |
| Bodily pain | 75.8 ± 19.5 |
| General health perceptions | 65.4 ± 15.8 |
| Vitality | 66.2 ± 20.4 |
| Social functioning | 89.4 ± 17.5 |
| Role-emotional | 89.5 ± 25.8 |
| Mental health | 75.7 ± 18.6 |
| Summary scales in SF-36 | |
| Physical components | 53.1 ± 6.9 |
| Mental components | 47.5 ± 9.6 |

Data are expressed as mean ± S.D.

hb PWV, 940.3 ± 157.4 cm/s for fa PWV. The means of lumbar spine BMD and calcaneus OSI were 0.876 ± 0.146 g/cm$^2$ and 2.54 ± 0.28 × 10$^6$, respectively.

### 3.2. Unadjusted domain scores of the eight subscales and adjusted domain scores of two summary scales of the SF-36 scores

Table 2 represents the HRQL scores assessed by SF-36. Adjusted domain scores of two summary scales, physical and mental components, were both around 50 points, indicating that the subjects enrolled in the present study have not been suffering from major health problems.

### 3.3. Correlations of IMT and PWV with clinical variables including lumbar spine BMD and calcaneus OSI

Table 3 shows the summary of correlations of IMT and PWV with clinical variables including lumbar spine BMD and calcaneus OSI by Spearman Rank correlation. Age, menopause, smoking index, systolic blood pressure, serum LDL cholesterol and triglyceride, but not HDL cholesterol and physical functioning score of SF-36, were positively correlated with CA and FA IMT. Lumbar spine BMD was negatively correlated with both CA and FA IMT, whereas calcaneus OSI was negatively correlated with FA IMT but not with CA IMT. Age, menopause, systolic blood pressure, serum LDL cholesterol and triglyceride were positively correlated with PWV of all segments. HDL cholesterol was negatively correlated with hc and hf but not hb and fa PWV. Smoking index was positively correlated with hf and fa PWV but not hc and hb PWV. Physical functioning score, lumbar spine BMD and calcaneus OSI correlated significantly in a negative fashion with all PWV.

Table 4
Multiple regression analysis of factors independently associated with IMT

| Independent variable | CA IMT | | FA IMT | |
|---|---|---|---|---|
| | Model 1 | Model 2 | Model 1 | Model 2 |
| Age | 0.261 | 0.252 | 0.598§ | 0.579§ |
| Menopause | 0.158 | 0.221 | −0.138 | −0.212 |
| Smoking index | 0.112 | 0.101 | 0.142 | 0.174§ |
| Systolic BP | 0.046 | 0.060 | −0.146 | −0.128 |
| LDL cholesterol | 0.200§ | 0.165§ | 0.019 | 0.058 |
| Lumbar spine BMD | 0.034 | – | −0.067 | – |
| Calcaneus OSI | – | 0.152 | – | −0.240§ |
| $R^2$ | 0.288‡ | 0.303‡ | 0.229‡ | 0.274‡ |

Values are standard regression coefficients (s). $R^2$: multiple coefficient of determination. BP: blood pressure, LDL: low-density lipoprotein, BMD: bone mineral density, OSI: osteo-sono-assessment index, CA IMT: common carotid artery–intima–media thickness, FA: femoral artery.
‡ $p < 0.0001$.
§ $p < 0.05$.

### 3.4. Multiple regression analysis of factors independently associated with IMT

Table 4 represents the results of multiple regression analysis of various clinical variables which correlated significantly in a simple regression analysis with CA and

Table 3
Correlations of IMT and PWV in each segment with clinical characteristics by Spearman Rank correlation

| Clinical variables | CA IMT | FA IMT | hc PWV | hf PWV | hb PWV | fa PWV |
|---|---|---|---|---|---|---|
| Age | 0.449‡ | 0.438‡ | 0.717‡ | 0.641‡ | 0.547† | 0.557‡ |
| Menopause | 0.549‡ | 0.519‡ | 0.725‡ | 0.650‡ | 0.605† | 0.606‡ |
| Smoking index | 0.301† | 0.311† | 0.262 | 0.253§ | 0.277 | 0.217§ |
| Systolic BP | 0.297† | 0.199§ | 0.588‡ | 0.638‡ | 0.627† | 0.678‡ |
| HDL cholesterol | −0.097 | −0.007 | −0.172§ | −0.167§ | −0.079 | −0.051 |
| LDL cholesterol | 0.328‡ | 0.277† | 0.336‡ | 0.322‡ | 0.266§ | 0.313§ |
| Triglyceride | 0.338‡ | 0.179§ | 0.333‡ | 0.372‡ | 0.242§ | 0.349‡ |
| Physical functioning score (SF-36) | −0.015 | −0.025 | −0.160§ | −0.157§ | −0.148§ | −0.192§ |
| Lumbar spine BMD | −0.225§ | −0.215§ | −0.343‡ | −0.283† | −0.289† | −0.281† |
| Calcaneus OSI | −0.051 | −0.330‡ | −0.235§ | −0.235§ | −0.170§ | −0.252§ |

Values indicate bivariate correlation coefficients (Spearman's rho) obtained from 149 healthy Japanese women. BP: blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein, BMD: bone mineral density, OSI: osteo-sono-assessment index, CA IMT: common carotid artery–intima–media thickness, FA: femoral artery, hc PWV: heart–carotid pulse wave velocity, hf: heart–femoral, hb: heart–brachial, fa: femoral–ankle.
‡ $p < 0.0001$.
† $p < 0.001$.
§ $p < 0.05$.