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ORIGINAL ARTICLE

Impact of *IL-22* gene polymorphism on human immunodeficiency virus infection in Han Chinese patients

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Background/Purpose: To analyze the polymorphism of the *IL-22* gene in Han Chinese patients and to evaluate the influence of *IL-22* polymorphism on human immunodeficiency virus (HIV) infection.
Methods: *IL-22* gene polymorphism was analyzed in 73 blood samples from healthy participants. The influence of the genotype and allele distribution of three single nucleotide polymorphisms (rs2227484, rs2227485, and rs2227513) of *IL-22* on HIV infection was evaluated in 619 HIV seropositive patients and 619 healthy controls. To determine the association between the rs2227513 genotype and IL-22 levels in plasma, we randomly selected 29 HIV seropositive blood samples and 15 healthy blood samples and measured the levels of IL-22.
Results: Nine single nucleotide polymorphism loci of the *IL-22* gene were found (rs2227484, rs2227485, rs2227491, rs2227508, rs2227513, rs1179249, rs1179250, rs1179251, and rs1182844). Stratified analysis (by sex) showed a higher association of HIV infection and the A/G genotype and G allele at rs2227513 in women, but not in men (A/G genotype odds ratio = 5.24, 95% confidence interval = 1.13–24.27; allele G odds ratio = 5.27, 95% confidence interval 1.15–24.23). The rs2227513 A/G genotype was also associated with significantly higher levels of plasma IL-22, regardless of whether the patient was HIV seropositive or seronegative.

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Conclusion: Our results suggest that IL-22 production in blood might act as a pathogenic factor in HIV infection.
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Introduction

China is a huge country with one-quarter of the world's population. The majority of the population (91.51%) is Han Chinese. China has been experiencing one of the most rapidly growing epidemics of human immunodeficiency virus (HIV) infection in the world over the past 20 years. By the end of 2011, >780,000 adults and children were estimated to be living with HIV infection, with an estimated overall HIV prevalence of 0.058% of the total population. High infection rates of HIV are concentrated among specific sub-populations, such as intravenous drug users, sex workers, and men who have sex with men.[1] Although there is still no effective vaccine and no cure for HIV infection and AIDS, viral susceptibility shows individual heterogeneity, which has suggested potential targets for treatment.[2] Patients who have maintained resistance to HIV infection after high-risk exposure have been documented in numerous cohorts from across the globe, including commercial sex workers and those practicing unprotected heterosexual or homosexual sexual intercourse with an HIV-infected partner.[3–5] The underlying mechanisms of individual heterogeneity are not fully explained, but many published studies have revealed a key role of genetic variation in the host.[6–8] From the early 1980s, genetic variants have been identified by using a combination of candidate gene analysis and unbiased genome-wide association scans; the results explain, in part, the observed variation in HIV susceptibility.

Interleukin-22 was first described in 2000 by Dumoutier et al.[9] It is a member of the IL-10 family and is produced by special immune cell populations, including specific T and NK cells, and lymphoid tissue inducer cells. The target cells of IL-22 are certain tissue cells from the skin, liver, and kidney, and from organs of the respiratory and gastrointestinal systems. Increased IL-22 expression has been observed in human infectious diseases, such as *Mycobacterium tuberculosis* infection and abdominal sepsis.[10–12] Moreover, IL-22 plays a part in host resistance to HIV infection.[13] Missé et al[14] carried out complementary transcriptome and proteome analyzes on blood samples from repeatedly HIV-exposed, uninfected patients and found increased blood IL-22 levels and increased IL-22 production *in vitro* via anti-CD3/anti-CD28-activated T cells. Several studies have explored the role of IL-22 in HIV infection and defense, with mixed results.[15–17] In this study, we analyzed the sequence polymorphism of the *IL-22* gene in Han Chinese participants and evaluated the influence of the genotype and allele distribution of three single nucleotide polymorphisms (SNPs) of *IL-22* on HIV infection in 619 HIV seropositive Han Chinese patients and 619 healthy Han Chinese controls. We also investigated the levels of IL-22 and its association with gene polymorphisms and HIV infection.

Materials and methods

Research protocols were approved by the institutional ethics review board of Sun Yat-Sen Memorial Hospital. Informed consent was obtained from all the participants before the samples were collected, in accordance with the Declaration of Helsinki.

Sequence polymorphism of *IL-22* gene

Sample collection

A total of 73 samples of peripheral venous blood from healthy participants attending for voluntary counseling and testing in Shenzhen Center for Disease Control and Prevention, Shenzhen, China were included in this study. All of the study participants (aged 39.2 ± 10.7 years, 37 men and 36 women) were from a Han Chinese population living in south China and had no direct relationships. Patients with HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), *Treponema pallidum* infection, inflammation, tumor, family history, or other serious diseases were intentionally excluded.

DNA sequencing

The genomic DNA of peripheral blood mononuclear cells of each participant was extracted from 200 μL of EDTA-anticoagulated peripheral blood samples with the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Duesseldorf, Germany) according to the manufacturer's protocol.

DNA sequencing was performed using a SangonBiotech (Shanghai) Co. Ltd ABI 3730xl DNA Analyzer (ABI Inc., Foster City, CA, USA). The sequencing reaction system included the purified polymerase chain reaction (PCR) production, primers for sequencing, Taq polymerase, and ABI PRISM BigDye terminators. The sequencing curve was analyzed by Chromas software version 2.33 (http://www.Technelysium.Com.au/chromas.html).

Two software packages, BioEdit version 7.0.9 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and CLUSTALX version 1.8 (flp://fip-igbmc.u.strasbg.fi-/pub/ClustalX/) were used to compile the sequence, matching the sequence multiple and confirming the potential SNP loci. The genotype of the SNPs was checked at least three times.

Impact of *IL-22* gene polymorphism on HIV infection

Participants

Six hundred and nineteen HIV-1 seropositive peripheral blood samples were collected in Shenzhen Center for Disease Control and Prevention from January 2005 to December 2009. The inclusion criteria for the HIV group were: (1) Han Chinese ethnicity; (2) the exclusion of *Treponema pallidum* infection; and (3) the exclusion of a

history of tumor. Six hundred and nineteen healthy Han Chinese participants were matched for age and sex. Table 1 shows the demographic parameters of the HIV-1 seropositive patients and controls.

Plasma IL-22

Plasma levels of IL-22 were measured by using a Human IL-22 Quantikine ELISA Kit (R&D System, Minneapolis, MN, USA) according to manufacturer's specifications. In brief, the wells were blocked with 100 μL/well of assay diluent and incubated for 2 hours at room temperature. The plate was then incubated for 2 hours at room temperature after the addition of 100 μL/well of supernatants and standards. IL-22 conjugate (200 μL/well) was added and incubated for 2 hours at room temperature. Each of these steps was interspersed with four washes. The wells were incubated with the substrate solution (200 μL/well) for 30 minutes at room temperature. The stop solution (50 μL/well) was added and absorbance was measured at 450 nm and subtracted by the absorbance values at 540 nm. Samples were assayed in duplicate.

Statistical analysis

The Hardy–Weinberg equilibrium test was performed by Haploview software version 4.2 (Broad Institute, Cambridge, MA, USA). The Hardy–Weinberg equilibrium linkage disequilibrium was analyzed using Haploview software (version 4.2); the haplotype reconstruction was analyzed by a Software Package for Phylogenetics And Sequence Evolution (PHASE) software (version 2.1) based on a Bayesian statistical approach. The genotype frequencies were compared by the Chi-square test or Fisher's exact test. The 95% confidence interval for the odds ratio parameter was calculated. The independent sample *t* test, one-way analysis of variance, and Lenene's test were used for the equal variances. Testing was performed at the 0.05 level of significance.

Results

Genotype distribution and allele frequency of *IL-22* SNPs

The *IL-22* gene sequence included five introns and six exons. The sequenced region of the *IL-22* gene included the majority of the exons, part of the introns, a 5′ untranslated region, and a 3′ untranslated region (Fig. 1). Nine SNP loci were found and there was no polymorphism in the coding region. rs2227484 and rs2227485 were located in promoter region; rs2227513 was located in the first intron area; rs2227491 was located in the third intron area; rs1179251, rs1179250, and rs1179249 were located in the fifth intron area; and rs2227508 and rs1182844 were located in the 3′ untranslated region. Haploview (version 4.2) was used to analyze the frequency of SNP alleles. The consistency of all the genotype frequencies was accorded with Hardy–Weinberg equilibrium.

Table 1 Demographic characteristics of HIV-1 seropositive patients and healthy controls

Parameter	HIV-1 seropositive patients (n = 619)	Healthy controls (n = 619)
Median (range) age	30 (17–73)	29 (17–68)
Male sex	442	442
Female sex	177	177
Heterosexual transmission[a]	318 (162:156)	—
Homosexual transmission[a]	130 (130:0)	—
Intravenous drug abuse[a]	147 (134:13)	—
Blood transmission[a]	24 (16:8)	—

[a] Data presented as *n* (male:female).

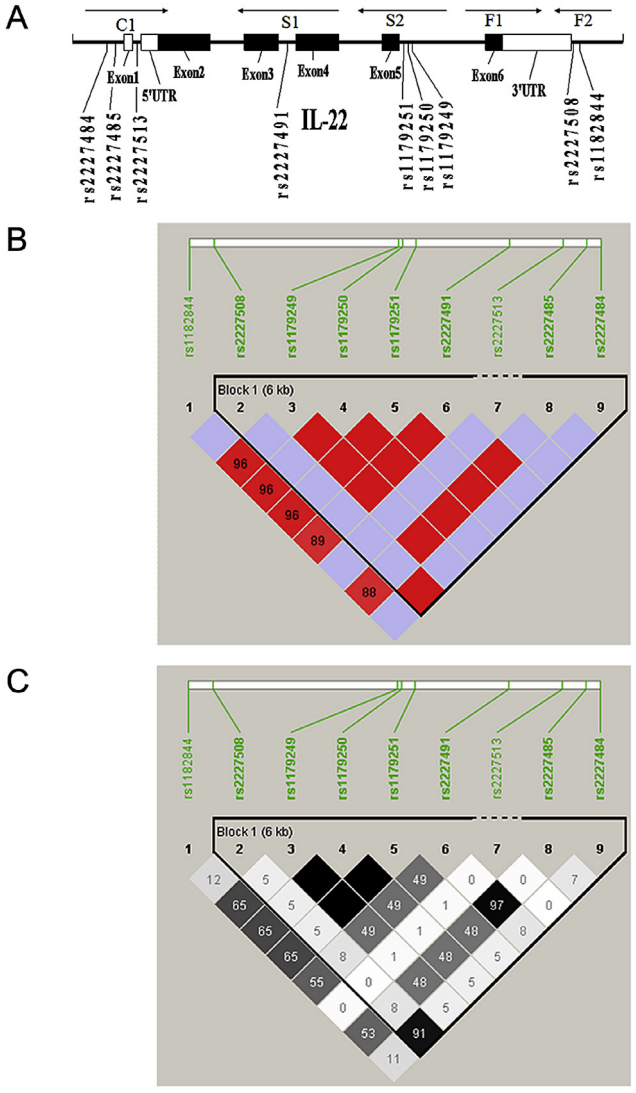

Figure 1. (A) Schematic diagram of the sequencing region for the *IL-22* gene. (B) Locus disequilibrium sketch map of standard colors for *IL-22* SNPs in Han Chinese participants. Different colors represent different ranges of D′ and LOD between two loci. Blue, D′ = 1, LOD < 2; shiny red, D′ = 1, LOD ≥ 2; white, D′ < 1, LOD < 2; light red, D′ < 1, LOD ≥ 2. (C) Locus disequilibrium sketch map of r^2 color for *IL-22* SNPs in Han Chinese participants. Different colors represent different ranges of r^2. White, $r^2 = 0$; gray, $0 < r^2 < 1$; and black, $r^2 = 1$.

Locus linkage disequilibrium among nine SNPs was analyzed with Haploview software (version 4.2). There was a haplotype block (Fig. 1), which included seven loci: rs2227508, rs1179249, rs1179250, rs1179251, rs2227491, rs2227485, and rs1182844.

Five taggers of the SNPs were screened according to the principle of $r^2 \geq 0.8$ and multi-marker tests limit of detection ≥ 3 from nine SNPs. There was no captured allele of the SNP locus because there was no strong site associated with rs2227513 and rs11828445. The mean of r^2 of these five tagger SNPs and the alleles captured SNPs is 0.988.

Genotype distribution and allele frequency of *IL-22* SNPs (rs2227484, rs2227485, and rs2227513) and the association with HIV infection

Nine SNPs of *IL-22* were identified in the Han Chinese participants. Among these, rs2227484, rs2227485, and rs2227513 were found in the putative promoter region. We analyzed the genotype and allele distribution of these three SNPs of *IL-22* in 619 HIV seropositive Han Chinese patients and 619 healthy Han Chinese controls. The allele frequency and Hardy–Weinberg equilibrium test for three SNPs in groups were comparable with the distributions analyzed in the 73 healthy participants.

The frequency of the C/T genotype and the T allele at rs2227484 in HIV seropositive patients infected via heterosexual transmission in men were significantly lower compared with the controls (C/T genotype frequency 6.8% vs. 16.1%, $p = 0.003$, odds ratio (OR) = 0.378, 95% confidence interval (CI) = 0.195–0.733; T allele frequency 3.4% vs. 8.7%, $p = 0.002$, OR = 0.368, 95% CI = 0.193–0.702; see supplementary data). The frequency of the C/T genotype and T allele at rs2227484 in HIV+/HBV−/HCV− patients were significantly lower as compared with that in control (C/T genotype frequency 8.9% vs. 16.1%, $p = 0.042$, OR = 0.505, 95% CI = 0.258–0.985; T allele frequency 4.4% vs. 8.7%, $p = 0.026$, OR = 0.486, 95% CI = 0.254–0.930; see supplementary data). These results indicated that the C/T genotype and T allele at rs2227484 were associated with a lower risk of HIV infection in these subgroups. In addition, the frequency of the C/T genotype and the T allele at rs2227484 in HIV and HBV and/or HCV seropositive patients in men were significantly lower compared with controls (C/T genotype frequency 0 vs. 16.1%, $p = 0.003$; T allele frequency 0 vs. 8.7%, $p = 0.003$; see supplementary data), which suggested that the C/T genotype and T allele at rs2227484 were associated with a lower risk of HIV and hepatitis virus co-infection in this subgroup.

The distribution of genotypes among patients with HIV infection and controls were comparable for rs2227485 and no significant association was observed (see supplementary data).

The frequencies of the A/G genotype and G allele at rs2227513 in HIV seropositive patients were significantly higher than in controls (A/G genotype: frequency 2.9% vs. 1.1%, $p = 0.026$, OR = 2.62, 95% CI = 1.09–6.32; allele G frequency 1.5% vs. 0.6%, $p = 0.027$, OR = 2.60, 95% CI = 1.08–6.23; supplementary data). Stratified analysis (by sex) showed an increased risk of HIV infection in relation to the A/G genotype and the G allele at rs2227513 in women but not in men (A/G genotype frequency 5.6% vs. 1.1%, $p = 0.035$, OR = 5.24, 95% CI = 1.13–24.27; allele G frequency 2.8% vs. 0.6%, $p = 0.02$, OR = 5.27, 95% CI = 1.15–24.23; see supplementary data).

Haplotype frequency distribution in *IL-22* promoter region and the association with HIV infection

Haplotype analysis was performed for HIV seropositive patients versus controls and four haplotypes of rs2227484-rs2227485-rs2227513 were observed. Compared with the C-T-A haplotype, the C-T-G haplotype was associated with a higher risk of HIV infection, particularly for women and heterosexuals (HIV seropositive patients vs. controls frequency 1.5% vs. 0.57%, $p = 0.029$, OR = 2.571, 95% CI = 1.066–6.202; HIV seropositive patients vs. controls (women) frequency 2.9% vs. 0.56%, $p = 0.021$, OR = 5.088, 95% CI = 1.099–23.566; HIV seropositive patients infected via heterosexual transmission vs. controls frequency 2.0% vs. 0.57%, $p = 0.004$, OR = 3.635, 95% CI = 1.435–9.205; HIV seropositive patients infected via heterosexual transmission vs. controls (women) frequency 2.6% vs. 0.6%, $p = 0.04$, OR = 4.494, 95% CI = 1.040–21.483; see supplementary data). The T-C-A haplotype was associated with a decreased risk of HIV infection in heterosexual men (frequency 3.4% vs. 8.6%, $p = 0.006$, OR = 0.406, 95% CI = 0.210–0.786, see supplementary data). In addition, the frequency of the C-T-G haplotype in HIV seropositive patients infected via drug abuse transmission was significantly higher compared with controls (frequency 7.7% vs. 0.6%, $p = 0.021$, OR = 17.3, 95% CI = 2.203–135.874; see supplementary data), indicating a higher risk of HIV infection in drug abusers with the C-T-G haplotype.

IL-22 plasma levels and rs2227513 polymorphism

To determine the association between the rs2227513 genotype and IL-22 levels in plasma, we randomly selected 29 HIV seropositive blood samples and 15 healthy blood samples and measured the levels of IL-22. The demographic parameters are shown in Table 2. Table 3 shows that the rs2227513 A/G genotype was associated with significantly higher levels of IL-22 than the A/A genotype, regardless of whether the patient was HIV seropositive or seronegative.

Discussion

Genetic factors alter the susceptibility to HIV in the human population and a particular group of people who are exposed to HIV may remained uninfected: "HIV-resistant" individuals.[6,18] In our study, the sequence polymorphism of the *IL-22* genes was analyzed and nine SNPs loci (rs2227484, rs2227485, rs2227491, rs2227508, rs2227513, rs1179249, rs1179250, rs1179251, and rs1182844) were found and verified in Han Chinese participants. We also evaluated the genotype and allele distribution of three SNPs of *IL-22* (rs2227484, rs2227485, and rs2227513) in 619 HIV seropositive Han Chinese patients and 619 healthy Han Chinese controls and investigated the IL-22 plasma levels and their association with gene polymorphisms and HIV infection. We

Table 2 Demographic characteristics of blood samples for IL-22 detection

Parameter	HIV-1 seropositive patients ($n = 29$)		Healthy controls ($n = 15$)	
rs2227513 genotype	A/G ($n = 18$)	A/A ($n = 11$)	A/G ($n = 7$)	A/A ($n = 8$)
Mean ± SD age*	29.61 ± 4.9	32.81 ± 5.1	29.85 ± 4.3	28.87 ± 4.4
Sex (male:female)	8:10	5:6	5:2	4:4

*$p = 0.287$.

found a higher association of HIV infection and A/G genotype and G allele at rs2227513, particularly in women. A further investigation of IL-22 levels in plasma showed that the A/G genotype at rs2227513 was associated with significantly higher levels of IL-22 than the A/A genotype, regardless of whether the patient was HIV seropositive or seronegative. These results suggest that IL-22 production in blood might act as a pathogenic factor in HIV infection.

IL-22 is a cytokine produced by immune cells and is important for the regulation of tissue cell responses during inflammation and infection. Many studies have described increased IL-22 production *in vivo*, including in inflammation and infection. The cellular sources of IL-22 in these studies varied depending on the location and nature of the affected tissue and the time of analysis. Increased IL-22 levels were found in several different chronic inflammatory conditions, including psoriasis, active Crohn's disease, and rheumatoid arthritis. IL-22 can have either a protective or a pathogenic role in chronic inflammatory diseases.[19–21] IL-22 also has a possible role in the innate immune defense against pathogens, e.g., bacteria and viruses. IL-22 regulates antimicrobial protein production and neutrophil recruitment and thus enhances the basic innate barrier defenses to enteric bacterial pathogens at mucosal surfaces.[22,23]

The role of IL-22 in antiviral defense is still controversial. Missé et al[14] found upregulated cell supernatant and plasma levels of IL-22 in repeatedly HIV-exposed, uninfected participants. However, Chege et al[15] concluded that HIV exposure without infection was associated with reduced production of IL-22, both systemically and at the site of mucosal HIV exposure. In this study, we found that there was an increased risk of HIV infection related to the A/G genotype at rs2227513. Further analysis showed an association between this polymorphism and significantly higher levels of IL-22, regardless of whether the patient was HIV seropositive or seronegative. Our results indicate a pathogenic role of IL-22 production on HIV infection and revealed the effect of genetic polymorphism in this process. With respect to the underlying mechanisms, studies have shown that IL-22 signaling, through its IL-22R receptor, plays a critical part in the promotion of antimicrobial immunity, inflammation, and normal barrier homeostasis. These include stimulating the secretion of antimicrobial proteins and enhancing tissue repair.[24] In repeatedly HIV-exposed, uninfected individuals, increased production of IL-22 by T cells is reflected in a specific increase in the secretion levels of the acute phase serum amyloid A (A-SAA). A-SAA plays a beneficial part in the host innate defense during the acute phase of inflammation and induces phosphorylation of the cellular HIV coreceptor CCR5 in myeloid immature dendritic cells. CCR5 phosphorylation results in a strongly decreased infection rate of immature dendritic cells during HIV exposure.[14] Moreover, some research has indicated that IL-22 and IL-17 may work in tandem to protect against HIV. IL-17 was found to be secreted by a number of RAR-related orphan receptor γt expression cells, including Th17 cells, $CD8^+$ $\alpha\beta$ T cells, $\gamma\delta$ T cells, lymphoid tissue inducer (LTi) cells, LTi-like innate lymphoid cells, invariant natural killer T cells, and mucosal-associated invariant T cells, which also secrete IL-22.[22,25] IL-17 augments inflammation in immune defense to extracellular pathogens and IL-22 shows tissue-protective effects, including reinforcing the tight junctions between enterocytes and enhancing epithelial cell proliferation. The IL-17—IL-22 axis was indicated to play a crucial part in maintaining mucosal immunity and barrier integrity.[23] The role of IL-22 in HIV infection and the underlying mechanisms are therefore not yet well understood. It will be important for further studies to examine the causal nature of the association and to define the cell subsets responsible for these differences.

In our study, the frequency of the C/T genotype and T allele at rs2227484 in HIV seropositive male patients infected via heterosexual transmission were significantly lower than in controls. Stratified analysis (by sex) showed an increased risk of HIV infection in relation to the A/G genotype and G allele at rs2227513 in women, but not in men. These results suggested sex differences in IL-22 SNP polymorphism in HIV infection. Evidence has shown that women and men are different in their susceptibility and response to infectious diseases. The mechanisms responsible for these sex-based differences include immunological pathways affected by sex hormones, differential expression of X-chromosome-encoded genes on immune responses to pathogens, as well as cultural and behavioral differences between the sexes.[26] With regard to sex differences in gene polymorphism, Schoultz et al[27] found combined polymorphisms in certain inflammation-related genes in the development of Crohn's disease in men, but not in women, suggesting a role of sex differences in the pattern of genetic susceptibility in the innate immune response.

We found that the rs2227513 A/G genotype was associated with significantly higher levels of IL-22 than the A/A

Table 3 Comparison of IL-22 levels between A/G and A/A genotypes at rs2227513

Genotype	No. of samples	IL-22 (pg/mL)	Mann—Whitney *U* test	*p*
A/G	25	30.63 ± 31.36	—	—
A/A	19	11.19 ± 7.43	69	<0.001

genotype, regardless of whether the patient was HIV seropositive or seronegative. rs2227513 resides in the first intron of the *IL-22* gene. It has been determined that intronic SNPs modulate gene expression.[28,29] Xu et al[30] found certain proteins were the major binding partner for the G-containing SNP site. The G-to-A transition weakens protein binding and facilitates exon skipping, leading to altered gene expression. Intronic SNPs may play a part by affecting microRNA-to-mRNA binding or through locus disequilibrium with another SNP.[31,32] However, how the rs2227513 intronic SNP of *IL-22* affects transcription and gene expression remains uncertain.

This study found and verified nine SNP loci of the *IL-22* gene in Han Chinese participants and an increased risk of HIV infection was associated with *IL-22* polymorphism. Further detection of IL-22 levels in plasma showed that the A/G genotype at rs2227513 was associated with significantly higher levels of IL-22 compared with the A/A genotype, regardless of whether the patient was HIV seropositive or seronegative. These results suggest that IL-22 production in blood might act as a pathogenic factor in HIV infection.

Conflicts of interest

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jmii.2014.09.002.