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今月のテーマ●ウイルス性肝炎の最新治療

B型肝炎ウイルスの変異と治療

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要旨：B型肝炎ウイルス遺伝子の変異をopen reading flame毎に概説した．preS-S領域においてはvaccine-induced escape mutant，PreS2領域の大きな欠失などが見られる．また，preC-C領域においてはHBe抗原の産生を低下させるプレコア領域および上流のbasic core promoter領域の変異が知られており，劇症肝炎や肝細胞癌との関連が示唆される．また，P領域の変異は各種核酸アナログ製剤の耐性株出現時に見られる変異である．

索引用語：vacccine-induced escape mutant，Occult HBV感染，HBe抗原，核酸アナログ

B型肝炎ウイルスは，その増殖の過程にRNAからDNAへの逆転写を含む．このため，他のDNAウイルスに比較してウイルス遺伝子の変異の頻度が高い．

遺伝子の変化がウイルス蛋白のプロモーター領域を始めとする転写調節領域に遺伝子変異がおきるとウイルス蛋白の発現量が変化する．また，アミノ酸変異により，ウイルス蛋白の読み取り枠（open reading flame；ORF）に変化がおこると，生成される蛋白の立体構造や抗原性に変化がおきる．こうした遺伝子あるいはアミノ酸の変異は，B型肝炎の病態や治療効果に影響を及ぼすことが知られている．ここではB型肝炎ウイルスのORF毎に遺伝子/アミノ酸変異に関して述べる．

I preS-S領域の変異

1．Vaccine-induced escape mutant

S遺伝子はHBVのエンベロープであるHBs抗原をコードする遺伝子である．HBs抗原の抗原性を決定するのに最も大切なのは“a determinant region”（AA124-147）と呼ばれる領域である．

この領域はS遺伝子の親水性領域である“major hydrophilic region（MHR）”（AA99-169）に含まれ，HBVの表面から突出する2つのループを作る（Figure 1）．したがってこの領域にアミノ酸変異がおきた場合，HBs抗原の構造に変化がおこる可能性がある．その代表が“vaccine-induced escape mutant”である．この変異株は，母子感染防止のためにHBワクチンの接種を受けた児の中に，HBs抗体陽性にもかかわらずHBs抗原陽性の例が見られたことからその存在が明らかにされた．最も有名なものはS遺伝子の145番目のアミノ酸（“a determinant region”内の2番目のループ上に位置する）がグリシンからアルギニンに置換された変異（G145R）である[1]．この変異があるとHBワクチンで誘導されたHBs抗体とHBs抗原との結合力が低下することが確認されている．G145Rが検出された児の母親からはG145Rが検出されないことから，G145Rはワクチン投与によって誘導されたものであると考えられる．

145番目のアミノ酸以外では，126番目，129番目，141番目にアミノ酸置換を有するものが

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Mutations in the hepatitis B virus and treatment of hepatitis

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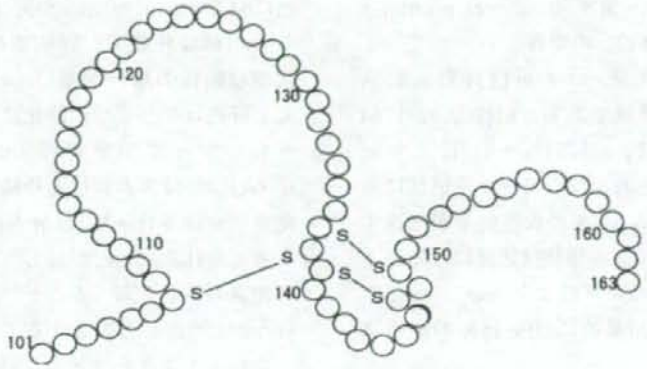

Figure 1. S遺伝子内の "a determinant" の構造

"vaccine-induced escape mutant" としてよく知られている[2][3].

2. PreS1 および PreS2 領域の変異

S領域の上流にはこれら2つの領域があり，S抗原には PreS1 から S までを含む "Large S protein"，PreS2 から S までを含む "Middle S protein"，S のみから生成される "Small S protein" の3種類がある．PreS1 領域の N 末端には，肝細胞表面への付着に必要な部位がある．また，C 末端には S promoterが存在する．したがって PreS1 領域はウイルスの増殖にとって大切な領域であり，変異は限られた部位にしか認められない[4].

PreS2 領域は，開始コドン部位に欠失や変異の入った株や大きな欠失を有する株が分離されている[4]．したがってウイルスの増殖や感染には必要不可欠な部位ではない．PreS2 領域の欠失は，B cell epitope や T cell epitope を消失させ，ウイルスに対する免疫応答を減弱させることから，ウイルスが生体の免疫応答から逃れるために変異したものと考えられる．

PreS2 領域の欠失は進展慢性肝疾患に多いことが最近台湾からの大規模な検討で明らかにされている[5][6]．preS2/S 蛋白は肝組織への組み込みから肝発癌へ関与している可能性が以前から指摘されており，関連のある可能性もある[7].

3. HBs 抗原陰性慢性肝疾患の症例における S 遺伝子変異

血清 HBs 抗原が陰性でありながら，HBV DNA が検出される症例は "Occult HBV 感染" と呼ばれる．HBV の変異により HBs 抗原の立体構造の変化がおこり，現行の検査では検出できない場合，HBs 抗原は見かけ上陰性となる場合がある[8]~[10]．また，HBV の複製や HBs 抗原の翻訳を阻害するような変異が生じた場合にも HBs 抗原は陰性となり得る[11]~[13].

II preC-C 領域の変異

1. プレコア領域（precore PreC：nt1814-1901）の変異

この領域の変異として最もよく知られているのは，nt1896 が G から A に変化することにより，28番目のアミノ酸がトリプトファン（TGG）から停止コドン（TAG）へと変化する変異である．この変異により，HBe 抗原の産生，分泌が不能となる．

Nt1896 の変異は HBe 抗体陽性の無症候性キャリアおよび慢性活動性肝炎患者で見られることが当初報告されたが[14][15]，その後，劇症肝炎患者でこの変異が高率に認められることが報告された[16][17].

Nt1896 の他には nt1899 などに変異をともなうことがある．

2. コアプロモーター領域（basic core promoter BCP：nt1742-1849）の変異

BCP領域はHBe抗原・コア蛋白質のmRNAの転写を制御する領域である．nt1762，nt1764の変異（nt1762 A→T，nt1764 G→A）により転写因子の結合が阻害され，プロモーター活性に影響が生じ，プレゲノムRNAの転写効率が亢進する．したがってウイルスの増殖は活発になる．また，nt1762，nt1764の変異によりpreC mRNAの翻訳は抑制され，結果的にHBe抗原の産生は低下する．

nt1762/1764の変異もpreC領域の変異同様劇症肝炎で高率に認められることが報告された[18][19]．

nt1762/1764の変異によりウイルス増殖能は約2倍に増加するが，さらにnt1753，nt1766，nt1768に変異が入った場合，ウイルスの増殖能は約8倍となる[20]．

HBe抗原の役割の1つは免疫応答の調節への関与だと考えられており，HBe抗原量が低下することにより，肝炎の病勢が強くなることが考えられる．B型急性肝炎では，変異型ウイルス（preCあるいはBCPの変異）の感染により，重症化・劇症化しやすいことが報告されている．しかしながら，上述のようにこれらの変異はHBe抗体陽性の無症候性キャリアでも見られることがわかっており，これらの変異のみで肝炎の重症化を説明するのは困難である．

3. preCおよびBCP領域の変異と慢性肝炎

preC領域nt1896およびBCP領域nt1762/1764の変異は慢性肝炎の自然経過とも密接な関連がある．

nt1896の変異はGenotype Bの症例ではGenotype Cの症例に比較して早期に検出されることが多い．nt1896が変異型，nt1762/1764が野生型の症例はGenotype Bで多く見られるが，この場合肝炎が早期に沈静化し，HBe抗体陽性の無症候性キャリアへと移行していくことが多い[21]．

Genotype Cの症例の多くでは，nt1762/1764の変異が早期におこり，nt1896の変異は遅れて出現する．nt1762/1764が変異型（nt1762 A→T，nt1764 G→A），nt1896が野生型の場合，ウイルスの増殖は旺盛で，肝病変の進展は速い．HBe抗原は陽性の場合が多い．nt1896が変異型になると肝炎は少しずつ沈静化に向かう[22]．

したがってBCP変異（nt1762 A→T，nt1764 G→A）は肝障害の程度と持続期間を反映し，preC変異（nt1896 G→A）は肝炎の沈静化を反映すると考えられる．したがって，肝炎増悪期にウイルス変異の各型を調べることで，抗ウイルス治療を行うかどうかの目安とすることが可能である．

なお，インターフェロン治療効果とBCP変異との間にも関連があるとされている[23][24]．

4. preCおよびBCP領域の変異と肝細胞癌

肝細胞癌の症例の大多数にはBCP変異（nt1762 A→T，nt1764 G→A）が認められる[25][26]．しかし，前述の通り無症候性キャリアにもこの変異は認められることを考えると，BCP変異は肝細胞癌発生のための必要条件に近いものと考えられる．

BCPおよびその上流の配列（転写調節に関与している）はX領域とoverlapすることもあり，発癌との関連がある可能性がある．本邦における解析からは，Genotype CのHBVキャリアの発癌例においては，非発癌例に比べ，1653番目および1753番目の変異が高率に認められることが示唆されている[27]．

5. コア領域の変異と慢性肝炎

HBe抗原陽性の症例の場合，無症候性キャリアに比べ，慢性肝炎例ではコア領域のアミノ酸変異が高率に認められる．特にAA84-101は超可変領域（hypervariable region）と呼ばれ，変異が集積している[28]．

これに対し，HBe抗体陽性の場合，慢性肝炎例に比較し，無症候性キャリアでコア領域のアミノ酸変異が高率に認められることが示唆されている[29]．

III ポリメラーゼ（pol）領域の変異

HBV pol遺伝子は（Figure 2）のような構造をしている．このうち，Pol/RT領域はHBVの逆転写酵素（reverse transcriptase）をコードする領域である．HBVはウイルス遺伝子の複製の際に，pregenomic RNAからウイルス遺伝子である

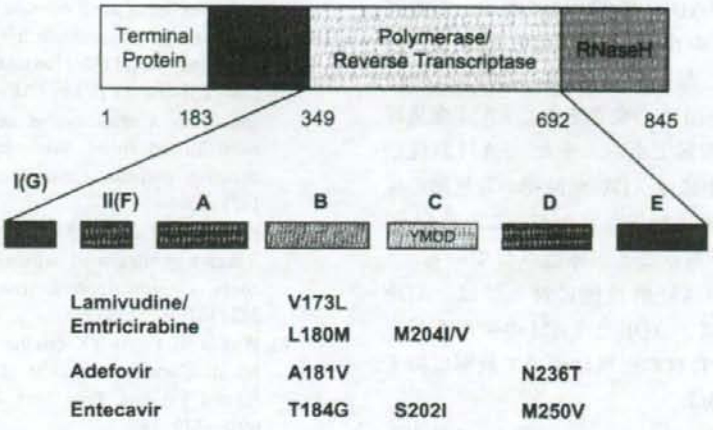

Figure 2. HBV ポリメラーゼ遺伝子の構造

Durantel D et al. J Clin Virol 2005 より

DNA への逆転写がおこる．この際に使われるのが逆転写酵素である．Pol/RT 領域はさらに Domain A から Domain E までの5ドメインに分かれている．Domain C には Pol/RT の活性中心があるとされている．Domain B，E は RNA テンプレートの結合に，Domain A，D は核酸との結合にそれぞれ重要とされている．

核酸アナログ製剤は逆転写の際に核酸の代わりに取り込まれてウイルスの複製を競合阻害するため，Pol/RT 遺伝子に変異が入った場合には薬剤耐性となる可能性がある．

主な核酸アナログ製剤に対し，耐性をもたらす，Pol/RT 遺伝子の変異を（Figure 2）に示す．

1．ラミブジン（LAM）に対する耐性

LAM 耐性の原因となるのは，Polymerase 領域の 204 番目のメチオニンがバリンあるいはイソロイシンに置換される変異（rtM204V/I）であり，YVDD 変異/YIDD 変異とも呼ばれる．また，rtM204V/I に随伴する変異として，180 番目のロイシンがメチオニンに置換される変異（rtL180M）が知られている．

rtM204M は HBe 抗原陽性 B 型慢性肝炎の場合，投与 24 週後で 10%，52 週後で 24%，5 年後で 65% に出現したと報告されている[30]．本邦における長期成績として，HBe 抗原陽性，陰性いずれの場合も投与5年後で 50% の耐性株出現率と報告されている[31]．

rtM204M を有する例のすべてに肝炎をともなうわけではない．肝炎のある症例ではポリメラーゼ遺伝子のアミノ酸変異が多いという報告があるものの，一定の変異が認められるわけではない．

最近，YMDD motif に変異がないにもかかわらず，LAM に耐性の症例が報告された．この症例では Pol 領域の 181 番目のアラニンがスレオニンに置換されており（rtA181T），キメラマウスを用いた実験により，この変異は LAM 耐性をもたらすことが証明されている[32]．

2．アデフォビル（adefovir dipivoxil）に対する耐性

Adefovir dipivoxil（ADV）は本邦ではラミブジン耐性が出現した症例に対し，ラミブジンとの併用で使用が許可されている．

ADV に対する耐性ウイルスの出現は，当初 ADV 単剤で検討が行われた．投与開始後3年で約 6% の症例に耐性が出現したと報告されている．耐性株には Pol 領域の 236 番目のアスパラギンのスレオニンへの置換（rtN236T），181 番目のアラニンのバリンへの置換（rtA181V），が認められている[33]．

一方，LAM 耐性が出現した症例に対し ade-

fovir dipivoxil (ADF) を投与した場合，投与開始後1年で18%の症例にrtA181V/TあるいはrtN236Tが出現したと報告されている[34]．rtA181TはYMDD motif内の変異を欠くLAM変異株にも認められる変異である．また，LAM耐性が出現した症例の中にはADVが最初から無効の症例があることが最近報告されたが，この場合rtI233Vを持つ株であることが確認されている[35]．しかしながら，LAM耐性例に対しては，ADF単独療法ではなく，ADFとLAMの併用を行うことで，ADF耐性株の出現は極めて低率に抑えることが可能である[36]．

3. エンテカビル (entecavir：ETV) に対する耐性

ETVを核酸アナログの治療歴のないB型慢性肝炎に対して使用した場合，ETV耐性となる頻度は極めて低いが[37]，LAM耐性例に使用すると48週投与で32%の症例に耐性ウイルスが出現したとする報告がある[38]．耐性ウイルスのRT領域のアミノ酸配列は2症例で解析されており，1症例ではrtM250V，rtI69Tが，もう1症例ではrtT184GおよびrtS202Iが認められている[39]．また，本邦からはrtS202G，rtL269Iが報告されている[39]．ETV耐性を獲得した症例は現在までのところ，いずれもLAM耐性の症例であるが，ETV単独で治療を開始した場合の薬剤耐性ウイルスの出現については今後検討が必要である．

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Persistent, Undetected *Trichomonas vaginalis* Infections?

To the Editor—In a recent large, randomized, controlled trial [1], 64 participants received a diagnosis of infection with *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, or *Trichomonas vaginalis* during intervals in which they reported not having sex. We considered the problems that might lead to this paradoxical situation, including errors in laboratory testing [2] and patient reporting [3] and treatment failure [4]. Our findings regarding *N. gonorrhoeae* and *C. trachomatis* infection were consistent with these scenarios; however, the findings regarding infection with *T. vaginalis* were surprising.

The HIV prevention trial was performed in 3 clinics that specialize in the treatment of sexually transmitted diseases [1]. At the initial visit, participants were counseled, examined, and tested for sexually transmitted diseases, including HIV infection. Infections were treated according to the treatment guidelines of the Centers for Disease Control and Prevention [4]. Outcomes were measured at 3, 6, 9, and 12 months after enrollment.

Nucleic acid amplification tests for *C. trachomatis* and *N. gonorrhoeae* infection were performed on urine specimens. *T. vaginalis* was cultured (from women only) using the InPouch TV test (BioMed Diagnostics) or modified Diamond's medium. Sensitivity has been estimated to be 82.4% for the InPouch TV test and 87.8% for culture on modified Diamond's medium; specificity for both culture methods is nearly 100% [5, 6]. At follow-up visits, vaginal swab specimens were obtained by the participant (at the Denver, CO, and Long Beach, CA, clinics) or a clinician (at the Newark, NJ, clinic).

Persons who reported having no sex partners were considered to have had no sex during that 3-month interval. Infections were considered to be new if the person had a negative test result at the beginning of the trial or had been treated for another infection at least 14 days before the infection was detected. Each participant could contribute up to 4 three-month intervals to the analysis. We measured associations with each new sexually transmitted infection by multivariate logistic regression models with generalized estimating equations with use of SAS (SAS Institute). [7].

Six hundred sixty-eight persons reported having had no sex during 1125 three-month intervals; 64 new infections were diagnosed among 59 of these persons during these intervals. Among persons who reported having no sex, test results were more likely to be positive for *T. vaginalis* (3.9%; the test for *T. vaginalis* had the highest reported specificity), compared with *N. gonorrhoeae* (1.4%; $P < .01$) or *C. trachomatis* (2.4%; $P = .1$).

The risk of new infection with *T. vaginalis* was nearly identical for women who did (4.2%) and did not (3.9%) have sex during the 3 months before receiving the diagnosis. New *T. vaginalis* infection was more likely to occur in women who were aged 26–39 years (6.2%), compared with women who were aged 16–25 years (2.0%; adjusted OR, 3.4; 95% CI, 1.2–9.5). Infection was more likely to occur in women who had a sexually transmitted infection at baseline (9.3%), compared with women who did not (2.0%; adjusted

Figure 1. *Trichomonas vaginalis* infections detected among women in intervals during which they were not having sex. Each row represents the history of 1 woman. Shaded areas are intervals during which the woman reported not having sex. Positive *(+)* and negative *(−)* test results for *T. vaginalis* are indicated for each woman.

OR, 6.2; 95% CI, 2.5–15.4). There was no increase in the risk of *T. vaginalis* infection among women who were infected with *T. vaginalis* during the immediately preceding interval (4.4%), compared with women who were not (3.9%). However, 13 (62%) of 21 new infections occurred in women who had been previously infected with *T. vaginalis*, and 11 (85%) of 13 had negative test results during the immediately preceding interval (figure 1).

Some of the women might have acquired infections during sexual contact that they did not report, and some might have had infections that were not detected at the baseline visit. However, many women were treated for infection, had negative test results, and then had positive test results again, which suggests that *T. vaginalis* was undetected by testing but still present for months after treatment. The possibility of long-term asymptomatic carriage is consistent with the age distribution of infected women; *T. vaginalis* is found more often in older women [8, 9]. This pattern is different from the pattern for bacterial sexually transmitted diseases but similar to that for incurable viral infections, such as herpes simplex virus type 2 [10]. Trials have suggested cure rates of >90%, but most have tested women once within a few weeks after treatment [11]. When women were tested again a few months after treatment, some of the previously cured women had infection detected again [11], and none of the studies continued testing women beyond a few months. Cultures might not detect infections if the concentration of *T. vaginalis* is low, which would be expected in asymptomatic infections [6, 12, 13]. Nucleic acid amplification tests may be better, but reports are inconsistent and the tests are not commercially available in the United States [14]. Similarly, self-obtained vaginal swab specimens occasionally miss infections, but the sensitivity of tests performed with self-obtained specimens has compared favorably with that of tests performed with clinician-obtained specimens [15].

Treatment failure could explain many of our findings, because 13 women had a documented preceding infection. However, our results were not simply attributable to treatment failure. Most of the women ($n = 11$) had an intervening negative test result before having a positive result during an interval when they reported not having sex. This suggests that, after treatment, *T. vaginalis* infection can become nondetectable for months and then reappear. Because these findings were unexpected and obtained with a small number of participants, additional studies are needed to confirm or refute these observations.

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Detection of HIV Type 1 Load by the Roche Cobas TaqMan Assay in Patients with Viral Loads Previously Undetectable by the Roche Cobas Amplicor Monitor

To the Editor—In March 2008, the Roche Cobas TaqMan assay replaced the Roche Cobas Amplicor Monitor, version 1.5, for measuring plasma HIV type 1 (HIV-1) load in Japan. This has resulted

in the detection of an HIV-1 load >50 copies/mL in some of the patients whose HIV-1 load had been undetectable (<50 copies/mL) by the Amplicor Monitor over several years and for whom antiretroviral treatment regimens had not been changed [1, 2].

A total of 1387 HIV-1–infected patients visited our outpatient clinic from March through June 2008, and their HIV-1 load was measured by the TaqMan assay. Among these patients, 876 regularly visited the clinic (once every 1–3 months) and had an undetectable HIV-1 load by the Amplicor Monitor at the last visit. Surprisingly, the TaqMan assay detected an HIV-1 load >50 copies/mL in 253 (28.9%) of the 876 patients, although antiretroviral treatment had not been modified for these patients. Furthermore, another 22 patients (2.5%) were found to have an HIV-1 load >40 copies/mL with use of the TaqMan assay. The same assay also detected HIV-1 RNA at levels lower than the linear range of the assay (<40 copies/mL) in 128 (14.6%) of the 876 patients.

We analyzed the relationship between TaqMan detectability and time during which the HIV-1 load was undetectable by the Amplicor Monitor. This time was defined as the period from the first HIV-1 load undetectable by the Amplicor Monitor to the viral load first measured by the TaqMan assay, without any HIV-1 load rebound or blip detected during the period. Interestingly, among the patients who had a viral load undetectable by the Amplicor Monitor for <1 year, 43.7% had an HIV-1 load >50 copies/mL detected by the TaqMan assay; among the patients who had a viral load undetectable by the Amplicor Monitor for >4 years, 18.5% had an HIV-1 load >50 copies/mL detected by the TaqMan assay (figure 1). Conversely, 37.3% of patients who had a viral load undetectable by the Amplicor Monitor for <1 year had HIV-1 RNA undetectable by the TaqMan assay, and 70.2% of patients who had a viral load undetectable by Amplicor Monitor for >4 years had an HIV-1 load undetectable by the TaqMan assay. Thus, the proportion of patients who had an HIV-1 load >50 copies/mL was inversely correlated with the duration that the viral load was undetectable ($R^2 = 0.895$), and the proportion of patients with undetectable viral load was positively correlated with the duration that the viral load was undetectable ($R^2 = 0.979$). These findings indicate that the longer the effective treatment, the greater the number of patients with HIV-1 RNA undetectable by the TaqMan assay.

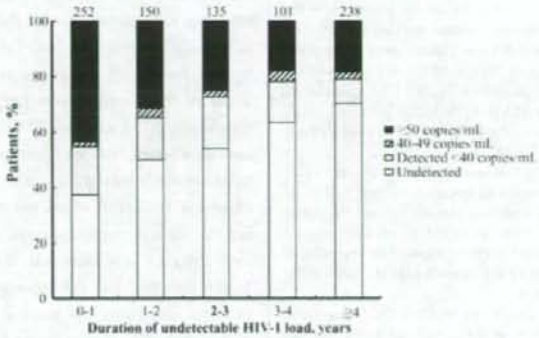

Figure 1. Results of the TaqMan assay and duration of undetectable HIV-1 load in 876 patients whose HIV-1 load was undetectable (<50 copies/mL) when the last Amplicor Monitor was performed. The number of patients is shown above each bar.

We observed significant discrepancy of HIV-1 detectability between the TaqMan assay and the Amplicor Monitor [3–5]. The TaqMan assay detected HIV-1 RNA in a significant percentage of treated patients with HIV-1 loads previously undetectable by the Amplicor Monitor; this is confusing to clinicians and patients and may be a critical problem in ongoing clinical trials of antiretroviral treatment. To determine the permissible range of detectable HIV-1 load during successful antiretroviral treatment, year-long clinical follow-up of treated patients is necessary. Our observation revealed that the detection rate of HIV-1 RNA with use of the TaqMan assay was inversely correlated with the previous duration of undetectable HIV-1 load, suggesting that long-term antiretroviral treatment can further suppress HIV-1 load even after it has decreased to below the detection limit of the Amplicor Monitor.

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Use of Active Surveillance Cultures in Intensive Care Units

To the Editor—I appreciated the systematic review by McGinigle et al. [1] of active surveillance cultures (ASCs) for methicillin-resistant *Staphylococcus aureus* (MRSA) in the intensive care unit (ICU) but question their conclusions about the lack of enough robust evidence to provide definitive recommendations for the use of ASCs in the control of MRSA infection. The authors included 20 studies, but only 13 of these studies seem to be original intervention studies that assess the effect of ASCs on the rate of MRSA infection. In addition, as the authors indicate, the methodology and/or robustness of many of these studies are not optimal.

Because I have been interested in this subject for many years, I have collected the literature on another 7 published nonpediatric and nonneonatal ICU studies that merit inclusion in the systematic review by McGinigle et al. [1–8], as well as another 6 neonatal and/or pediatric ICU studies (not referenced). It would be interesting to understand why these adult ICU studies were not included in the systematic review by McGinigle et al. [1]. Three of these studies were interrupted-time series, and 1 was a controlled before-and-after study; both of these methodologies are fairly robust. It is true that not all of the studies included weekly ASCs, but this seems to be a questionable exclusion criteria if a reduction in the rate of MRSA infection was still reported. However, the consistency of positive findings in the adult ICU studies is worth emphasizing (i.e., ASCs can aid in the control of MRSA infection in the ICU, particularly when ASCs are combined with at least 1 of the following: patient and environmental decontamination and hand-hygiene initiatives).

It is noteworthy that, of the 20 studies (13 in the systematic review and the 7 aforementioned adult ICU studies), only 3 do not mention use of additional hygiene and/or decontamination procedures (4 of 26 studies, if the neonatal and/or pediatric ICU studies are also considered). Moreover, although all but 1 study reported a reduction in the rate of MRSA infection after introduction of ASCs, this 1 study was notable for its poor hand-hygiene compliance, late isolation of MRSA-positive patients, and absence of any decontamination or disinfection.

Finally, the rating of high-quality interrupted-time series as only "fair" evidence by McGinigle et al. [1] is debatable. The most important difference between my interpretation of the data and that of McGinigle and colleagues is my observation of the consistency, strength, temporal relationship, and plausibility of the evidence; this insight led me to conclude that ASCs should be recommended as standard practice, particularly in high-risk areas, such as ICUs, where there is a high rate of hospital-acquired MRSA infection and a great risk of MRSA infection.

Incidentally, my colleagues and I conducted a study [9] (which was incorrectly referenced in the systematic review) that demonstrated a two-thirds reduction in the rate of MRSA infection (a decrease from >15% to ~5% of ICU admissions, not the 11% reduction stated in the systematic review by McGinigle et al. [1]). Moreover, this reduction was entirely attributable to a reduction in the number of MRSA isolates from clinical specimens, not screening specimens. Although the number of MRSA isolates is only a surrogate for infection, it is more closely indicative of infection than colonization; that the number of MRSA isolates is a surrogate marker of colonization was wrongly implied by Milstone and Perl [10] in their accompanying editorial commentary. In support of the number of MRSA isolates being a surrogate marker of infection, there was a significant reduction in both length of stay and glycopeptide use associated with the introduction of ASCs.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

Clinical and Radiological Features of *Pneumocystis* Pneumonia in Patients with Rheumatoid Arthritis, in comparison with Methotrexate Pneumonitis and *Pneumocystis* Pneumonia in Acquired Immunodeficiency Syndrome: A Multicenter Study

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Abstract

Objective To elucidate the clinical and radiological features of *Pneumocystis* pneumonia (PCP) in patients with rheumatoid arthritis (RA), compared with methotrexate (MTX) pneumonitis in RA and *Pneumocystis* pneumonia in acquired immunodeficiency syndrome (AIDS).

Subjects and Methods Retrospective analysis of 14 PCP cases in RA (RA-PCP), 10 MTX pneumonitis cases in RA (MTX-P) and 11 PCP cases in AIDS (AIDS-PCP) from 9 centers in the Kanto area in the last 6 years.

Results Compared with AIDS-PCP, both RA-PCP and MTX-P developed more rapidly, showing higher serum CRP and lower plasma β-D-glucan levels, and more severe oxygenation impairment. In most of the RA-PCP cases, a high dose of corticosteroid was administered as adjunctive therapy, resulting in a favorable outcome. The mortality was 14% in RA-PCP, 0% in AIDS-PCP and 0% in MTX-P cases. In RA-PCP patients the CD4 cell count showed only mild suppression, not reaching the predisposing level for PCP in HIV infection, suggesting that there are risk factors for RA-PCP other than immunosuppression. Radiologic analysis revealed some characteristic patterns of each disease. In MTX-P, diffuse homogeneous ground glass opacity (GGO) with sharp demarcation by interlobular septa (type A GGO) was found in 70%, while in AIDS-PCP diffuse, homogeneous or nonhomogeneous GGO without interlobular septal boundaries (type B GGO) was predominant (91%). In RA-PCP, type A GGO was found in 6 cases and type B GGO in 5 cases, showing the complex nature of this disease.

Conclusion RA-PCP differed considerably from AIDS-PCP clinically and radiologically. Clinically it occurred without severe immunosuppression, and showed characteristic aspects, with more intense inflammation and less parasite burden. Radiologically it mimicked MTX-P in some cases sharing the conspicuous CT features of MTX-P, rendering the distinction of these two disorders difficult.

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Key words: rheumatoid arthritis, *Pneumocystis* pneumonia, methotrexate pneumonitis, β-D-glucan, CT, acquired immunodeficiency syndrome (AIDS)

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Introduction

Pneumocystis pneumonia (PCP) is one of the uncommon but serious, life-threatening complications in patients with rheumatoid arthritis (RA) receiving treatment with methotrexate (MTX) (1-3). However it is often difficult to establish a definitive diagnosis, because the clinical and radiological presentations closely resemble those of MTX induced pneumonitis (MTX-P). Both are characterized by acute, progressive respiratory symptoms and diffuse bilateral infiltrates on chest radiography. The clue enabling a distinction lies in the detection of *Pneumocystis jirovecii* (*P. jirovecii*). However it is well known that traditional staining is often not sensitive enough in PCP in non-HIV conditions (4). Recently polymerase chain reaction (PCR) has been widely used for detection of this organism, with satisfactory sensitivity (5-7), but this method alone has the problem of false-positivity (8, 9). The subsidiary role of serology, especially measurement of β-D-glucan, has not received much attention.

We conducted a retrospective multicenter study to elucidate the clinical and radiological characteristics of RA-PCP, comparing it with MTX-P and also with AIDS-PCP , in order to discuss the problem of the differential diagnosis of these diseases.

Materials and Methods

Fourteen cases of PCP during treatment for RA were identified at 7 participating centers in Tokyo and its suburbs by practicing rheumatologists or pneumologists from April 2001 to August 2006. Ten cases of MTX-P were also identified at these centers during the same period. For comparison with RA-PCP, 11 cases of AIDS-PCP were randomly selected at two AIDS centers in Tokyo from March 2001 to December 2005. All of these cases were enrolled in the study after confirming that they had sufficient clinical information and imaging materials obtained before the beginning of definitive treatment for pulmonary events. Among them, 32 cases had thin section CT images of less than 2 mm collimation, while the other 3 cases had CT images using 5 mm collimation, both of good quality.

A diagnosis of PCP (both in RA and AIDS) was based on satisfaction of all of the following criteria; a) symptoms such as fever, cough and progressive dyspnea, associated with diffuse bilateral infiltrates on chest radiography, b) detection of *P. jirovecii* by traditional staining (Grocott or Diff-Quik or Giemsa staining) or by PCR in respiratory specimens, c) significantly elevated plasma (1→3)-β-D-glucan (β-D-glucan) level.

β-D-glucan was measured either with the β-glucan test WAKO (Wako Pure Chemical Industries, Tokyo, Japan) or with the FUNGITEC G test MK (Seikagaku Corp., Tokyo, Japan).

MTX-P was diagnosed based upon the same clinical presentations mentioned above and exclusion of infection, especially PCP, through intensive diagnostic procedures such as bronchoscopy or examination of sputum and measurement of plasma β-D-glucan. Clinical improvement following corticosteroid therapy was also taken into account.

The clinical background and preceding disease course of each patient was assessed with special attention to the dose and duration of antirheumatic drugs and also to the underlying disease. Clinical data at the recognition of the event, the clinical course and its outcome were evaluated.

Chest radiography and computed tomography (CT) were reviewed by two diagnostic radiologists. CT findings were categorized into three patterns: a) diffuse ground glass opacity (GGO) distributed in a panlobular manner, that is, GGO was sharply demarcated from the adjacent normal lung by interlobular septa (type A GGO) (Fig. 1A, Fig. 1B), b) diffuse GGO homogeneous or somewhat not homogeneous in distribution but without sharp demarcation by interlobular septa (type B GGO) (Fig. 2A, Fig. 2B), c) another pattern such as mixed consolidation and GGO (type C) (Fig. 3). The occurrence of each pattern was assessed in each group. The clinical features of each group and also their relationship with CT patterns were analyzed statistically using the Mann-Whitney-U test or Fisher's exact test.

Results

Patient characteristics

Table 1 shows the epidemiologic features of these patients. The RA-PCP group consisted of 14 patients, 2 men and 12 women, and they had a mean age of 66.5 years. *P. jirovecii* was detected in bronchoscopic specimens in 5 cases, in sputum examination in 9, by traditional staining in 3, by PCR in 11 cases. RA had been diagnosed for 11 years (mean). All had a history of receiving corticosteroid therapy and 13 patients were receiving MTX therapy (mean duration of 36.3 months) at the evolution of the lung events. Four patients were concomitantly receiving anti-TNF agents (three cases infliximab and one case etanercept). Six patients had

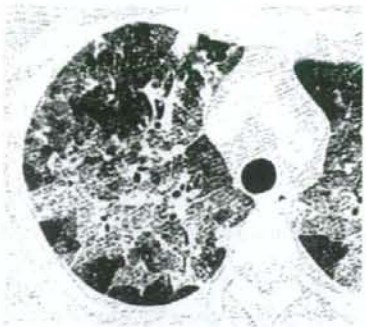

Figure 1A. **Type A ground glass opacity (GGO): GGO sharply demarcated from adjacent normal lung by interlobular septa. Methotrexate pneumonitis (MTX-P) was revealed in a 57-year-old woman who had received MTX therapy for 9 years for rheumatoid arthritis (RA). CT image shows homogeneous GGO which is clearly demarcated from adjacent normal lobules by interlobular septa.**

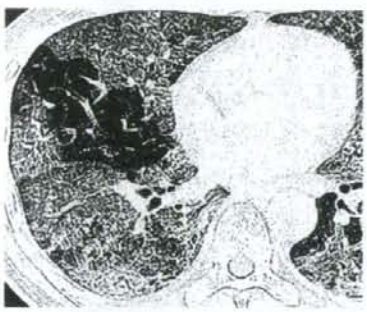

Figure 1B. **Type A GGO. A case of MTX-P in a 61-year-old man. He had received MTX therapy for 7 years. He had severe respiratory distress on admission, was treated with mechanical ventilation and resulted in favorable outcome. CT shows homogeneous GGO sharply demarcated from non-affected lung by interlobular septa.**

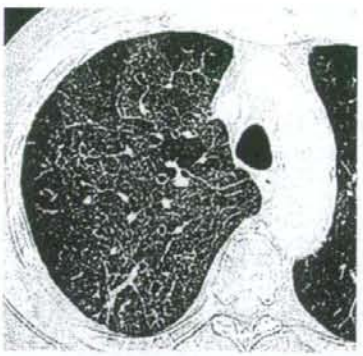

Figure 2A. **Type B GGO: homogeneous or nonhomogeneous GGO without sharp demarcation. An 83-year-old man had been treated for RA for 9 years with prednisolone (PSL) and MTX. MTX-P was diagnosed through exclusion of infection with bronchoscopy. CT shows nonhomogeneous GGO without sharp demarcation.**

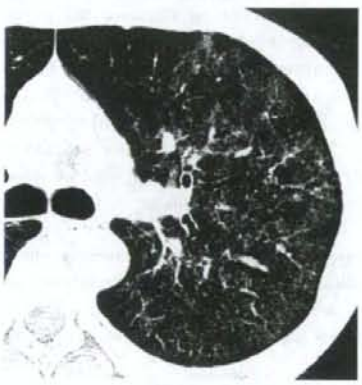

Figure 2B. **Type B GGO. A 53-year-old man had been diagnosed as HIV positive for 6 years. *Pneumocystis* pneumonia (PCP) was confirmed through positive staining for *Pneumocystis jirovecii* (*P. jirovecii*) in his sputum. CT shows diffuse, nonhomogeneous GGO without obvious demarcation.**

chronic interstitial lung disease (ILD) defined by the presence of honeycombing in CT.

The AIDS-PCP group included 11 cases, 10 men and 1 woman, with a mean age of 39.8 years. All were seropositive for the human immunodeficiency virus (HIV) antibody. *P. jirovecii* was detected by traditional staining in 5 cases and by PCR in 9.

The MTX-P group included 10 patients, 3 men and 7 women, had a mean age of 67.4 years. The diagnosis was made through exclusion of infection, especially PCP, by negative staining or PCR for *P. jirovecii* in 11 cases, and by low plasma β-D-glucan level in 2 cases. They had suffered from RA for 12 years (mean), and 7 of them had a history of corticosteroid therapy. MTX had been given for a duration of 31.0 months (mean). Two patients were concomitantly receiving anti-TNF agents (one case infliximab only once, one case etanercept). None of the patients of this group had ILD.

Clinical features

The clinical features of these three groups are shown in Table 2. Fever, cough and progressive dyspnea were predominant symptoms among all three groups. These symptoms preceded the diagnosis of the event with a period of 8.0±6.0 days in the MTX-P group, 7.6±6.4 days in the RA-PCP group, and 37.9±24.3 days in the AIDS-PCP group. The disease development was significantly faster in the MTX-P and the RA-PCP groups than the AIDS-PCP group. The serum CRP level was significantly higher in RA-PCP and MTX-P group than AIDS-PCP group (Fig. 4). The

plasma β-D-glucan level of AIDS-PCP was significantly higher (965.4 pg/ml, mean) than that of RA-PCP (98.5 pg/ml, mean). The value was below the cut-off level in MTX-P cases.

The CD4 cell count was 780.0±497.1/μl in the MTX-P group, 793.2±274.8/μl in the RA-PCP group, and 62.9±79.5/μl in the AIDS-PCP group, respectively. Taking the preserved serum immunoglobulin G (IgG) level into account, RA-PCP patients, as with MTX-P patients, showed a slight to moderate degree of immunosuppression, which was markedly different from AIDS-PCP patients (Fig. 5). PCP is usually considered to be an opportunistic infection under immunosuppressed conditions, but the immunological status was not greatly impaired in RA-PCP group. Severe hypoxemia necessitating oxygen supplementation was seen in 8 (80%) MTX-P cases, 11 (78.6%) RA-PCP cases and 3 (27.8%) AIDS-PCP cases. In summary, RA-PCP patients, along with MTX-P patients, showed more rapid clinical development, had significantly higher CRP level, lower β-D-glucan level, and worse oxygenation than AIDS-PCP patients.

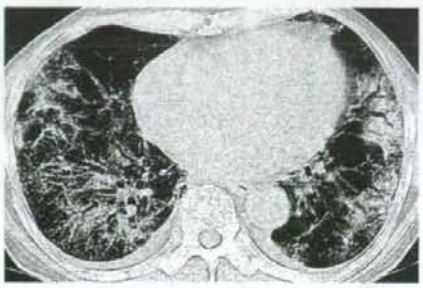

Figure 3. Type C : other type, mixed GGO and consolidation. A 69-year-old man was given a diagnosis of MTX-P. CT shows GGO intermingled with multiple foci of consolidation.

Patient outcome

All RA-PCP patients were treated with Trimethoprim-Sulfamethoxazole (TMP-SMX), together with corticosteroids (pulse therapy using methyl-prednisolone 500-1000 mg/day for 3 days in 4 cases, pulse therapy+oral prednisolone in 9 cases and oral prednisolone in 1 case). Eleven cases needed oxygen supplementation but none required mechanical ventilation. Two cases died despite intensive treatment, while the other 12 cases recovered completely within 3 or 4 weeks after admission (Table 2).

Eleven cases of the AIDS-PCP cases were treated with TMP-SMX. Adjunctive corticosteroids were given in 5 cases (oral prednisolone for 2 weeks). Three cases needed oxygen supplementation but none required mechanical ventilation. All patients recovered.

All MTX-P patients received steroid pulse therapy followed by 30-60 mg/day oral prednisolone as an initial dose with tapering. Although two cases required mechanical ven-

Table 1. Patient Characterstics

	MTX-P	RA-PCP	AIDS-PCP
number	10	14	11
male:female	3; 7	2;12	10; 1
age‡	67.4(46~88)	66.5(52~80)	39.8(29~58)
Detection of *P jirovecii* organism			
bronchoscopy	(7)†	5	4
sputum		9	7
traditional staining		3(Grocott 1, Diff-Quik 2)	5(Grocott 5, Diff-Quik 3)
PCR		11	9
duration of RA(years)‡	12(8-28)	11(1-26)	
Corticosteroids user	7	14	none
Methotrexate user	10	13	none
Mtotrexate duration(mo)‡	31.0(4~104)	36.3 (1 to 78)	
anti-TNF agents	1 infliximab, 1 ethanercept	3 infliximab, 1 ethanercept	none
lung comorbidity	0	6 chronic ILD	0

† done and resulted in negative study

‡ data are shown at median (with range)

abbreviations: MTX-P = methotrexate pneumonitis, RA-PCP = *Pneumocystis* pneumonia in rhuematoid arthritis, AIDS-PCP = *Pneumocystis* pneumonia in AIDS, AIDS = acquired immunodeficiency syndrome , *P jirovecii* = *Pneumocystis jirovecii* , Grocott = Grocott methenamine silver staining, Diff-Quik = Diff-Quik staining, PCR = polymerase chain reaction

Table 2. Clinical Features

	MTX-P(n=10)	RA-PCP(n=14)	AIDS-PCP(n=11)
duration of symptoms(days) before diagnosis	8.0±6.0	7.6±6.4	37.8±24.3
cough	5(50%)	5(42%)	7(64%)
fever	4(40%)	9(75%)	8(73%)
dyspnea	8(80%)	10(83%)	6(55%)
Alb (g/dl)	2.95±0.43	3.20±0.43	3.36±0.46
LDH (IU/l)	427.1±158.5	435.1±141.6	430.4±150.0
CRP (mg/dl)	11.6±6.2	8.6±4.8	2.3±2.2
KL-6 (U/ml)	814.3±757.5	1204.0±827.0	2490.8±1853.3
β-D-glucan (pg/ml)	below cut off level	98.5±94.8	969.5±1064.6
Leukocyte count(/μl)	7913.3±1851.8	8126.4±3284.3	7154.5±3433.4
Lymphocyte count (/μl)	1096.3±792.9	1028.7±599.6	963.2±684.9
CD4 cell count (/μl)	780.0±497.1	793.2±274.8	62.9±79.5
IgG (mg/dl)	1551±367	1056±340	n.d.
O_2 supplementation needed	8(80%)	11(78.6%)	3(27.8%)
ventilater needed	2(20%)	0	0
use of adjunctive corticosteroids	10(100%)	10(71.4%)	5(27.2%)
outcome(number of death)	0	2(14.3%)	0

* data are presented at median(with standard deviation)

abbreviations : Alb = serum albumin, LDH = lactate dehydrogenase, CRP = C-reactive protein, β-D-glucan = (1→3)-β-D-glucan, IgG = immunogloblin G

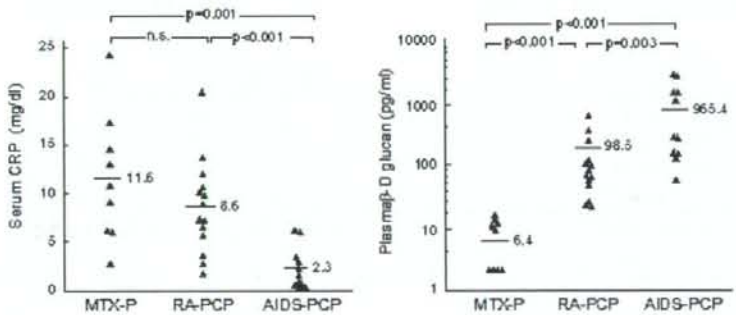

Figure 4. Serum CRP and plasma β-D-glucan in the three groups. CRP is significantly higher in MTX-P and *Pneumocystis* pneumonia in RA patients (RA-PCP) than *Pneumocystis* pneumonia in AIDS patients (AIDS-PCP), while β-D-glucan is significantly lower in RA-PCP than AIDS-PCP.

tilation, all recovered well.

Radiologic features

All patients showed diffuse bilateral infiltrates on chest radiography which, by itself, is neither specific nor pathognomonic for any of these three disorders. Through the analysis of CT images, we found three patterns of opacities, as mentioned above. The occurrence rates of these three patterns in each group are shown in Table 3. In the MTX-P group, the type A pattern predominated, noted in 7 cases (Fig. 1A, Fig. 1B), while type B was found in 2 (Fig. 2A), and type C in 1 case (Fig. 3). Type A was the most predominant image pattern for MTX-P. On the other hand, in the AIDS-PCP group, type A was found only in 1 case,

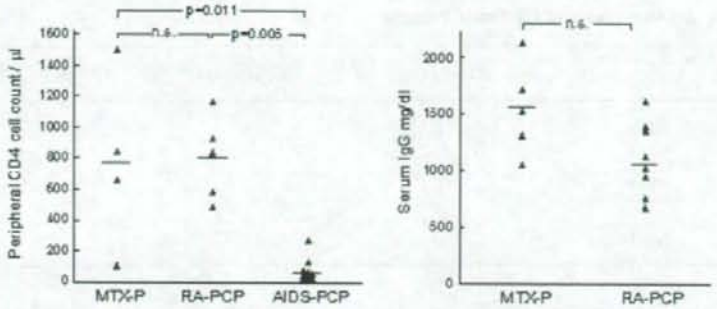

Figure 5. Immunological status of each group represented by peripheral CD4 cell count (measured in every group) and serum IgG (not measured in AIDS-PCP group). Both RA-PCP and MTX-P show a relatively preserved immunological condition in contrast with AIDS-PCP.

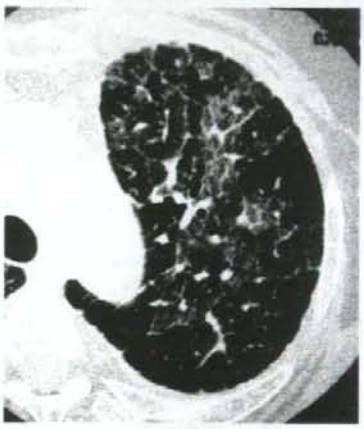

Figure 6A. GGO seen in a RA-PCP patient. A 71-year-old woman had received MTX therapy for 6 years. PCP was diagnosed based on elevated β-D-glucan and positive PCR for *P. jirovecii* in bronchoalveolar lavage fluid. CT shows type A GGO.

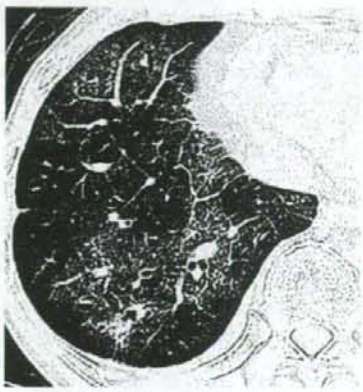

Figure 6B. GGO seen in a RA-PCP patient. A 64-year-old man had received MTX therapy for 5 years. *P. jirovecii* was identified with Grocott staining with marked elevation of serum β-D-glucan. CT shows type B GGO, nonhomogeneous pattern without lobule to lobule demarcation.

while the other 10 cases showed type B (Fig. 2B), suggesting type B to be the typical image pattern of this disease. Among the RA-PCP group, 6 cases showed type A pattern (Fig. 6A), 5 cases showed type B (Fig. 6B), and three cases type C, showing the complex nature of this disorder. The occurrence of type A GGO in RA-PCP did not differ significantly from that of MTX-P. We analyzed the relationship of these image patterns in CT and clinical features, but failed to find any relevance (data not shown).

Discussion

MTX is now widely used for the treatment of RA, because of its efficacy and low toxicity. In association with the increased use of MTX, serious and life-threatening lung complications have been increasingly reported (1-4, 10-12). One is PCP and another is MTX-P. Both diseases develop acutely and may sometimes result in serious consequences. PCP is an infectious disease in an immunosuppressed condition and should be treated with antimicrobial agents. MTX-P is a hypersensitivity reaction and should be treated by withdrawal of MTX, often followed by corticosteroids. To distinguish between these two conditions, RA-PCP and MTX-P, is therefore very important in the clinical context of acute onset lung injury during the treatment for RA with MTX.

The distinction, however, is often very difficult to make because of their similar clinical presentations. Imaging features are also so similar that no definitive difference has been reported between the two. Above all, the detection of *P. jirovecii*, which is mandatory for the diagnosis of PCP, is often very difficult in RA-PCP patients. In PCP patients without AIDS such as those of connective tissue disorders (CTD) receiving immunomosuppresive therapy (13), it is well documented that the organism numbers of *P. jirovecii* are significantly fewer in respiratory specimens (14-17). In

Table 3. Occurrence of CT Image Patterns

	MTX-P (n=10)	RA-PCP (n=14)	AIDS-PCP (n=11)
type A	7	6	1
type B	2	5	10
type C	1	3	0

type A, type B, type C : see text

such a situation, traditional staining is often not sufficiently sensitive. PCR for *P. jirovecii* is a much more sensitive technique than traditional staining (5) and its usefulness in the diagnosis of PCP, especially with low organism burden, was reported by many investigators (4, 6, 7). On the other hand several studies found incontrovertible incidence of colonization of *P. jirovecii* among immunosuppressed patients, suggesting that a positive PCR result alone may lead to overdiagnosis (8, 9). Meanwhile the measurement of β-D-glucan, a quantitative marker for mycotic diseases, has been reported as a useful and reliable marker in the diagnosis of PCP (18-21). Thus, we considered that, for the diagnosis of RA-PCP, detection of *P. jirovecii* by traditional staining is desirable but cases of positive PCR results with negative smears are also eligible when the plasma β-D-glucan level is significantly elevated.

Radiologic features of MTX-P

The radiologic features of MTX-P have been reported by many authors (10-12). They have been noted only as diffuse infiltrates on radiography and GGO on CT, with no further details described. Through the analysis of CT images of our cases, we found conspicuous features of MTX-P, that is, type A GGO as the predominant pattern on CT, which has never been reported.

RA-PCP compared to AIDS-PCP

Several important differences were found between the two groups clinically and radiologically. RA-PCP developed more rapidly than AIDS-PCP. Respiratory impairment was more severe in RA-PCP, and resulted in two deaths, while there was no fatality in the AIDS-PCP group. The level of plasma β-D-glucan, a quantitative marker for *P. jirovecii*, was significantly lower compared to AIDS-PCP, suggesting a lower organism burden in RA-PCP cases. All these differences have been well documented in many studies as the differences of PCP in patients with and without AIDS (13-17). Limper et al conducted a clinicopathological and comparative study of PCP of both conditions, including quantitative assay of *P. jirovecii* and inflammatory cells in BAL fluid, demonstrating fewer parasite numbers and more intense lung inflammation and also severe clinical symptoms in non-HIV PCP (15).

It is noteworthy that in our RA-PCP patients, the immunological status was not impaired as severely as in AIDS-PCP patients. These facts, i.e., relatively preserved immunity in RA-PCP patients, have been pointed out in several reports (22, 23). Why PCP can occur in patients who are not severely immunosuppressed is a problem to be solved, especially in relation to some particular immunomodifying actions of anti-rheumatic drugs.

The radiologic features of AIDS-PCP have been extensively reported (24-26), but not as thoroughly for non-AIDS-PCP or for the difference between the two, PCP with and without AIDS. Through detailed radiologic analysis, we found differences between these two disorders, which have apparently never been documented previously. In most AIDS-PCP cases, CT presented type B GGO. We consider this finding, which coincides with features previously reported (26-28), to be characteristic of this disease. However, in 6 of the 14 RA-PCP cases, CT showed type A GGO, while 5 presented type B GGO. RA-PCP showed complex radiological findings, intermediate between AIDS-PCP and MTX-P. Since the radiologic features might reflect the pathophysiology of each disease, we conducted a comparative analysis of the CT patterns and the clinical features of each disease, but failed to demonstrate any correlation, either with the clinical features or with patient outcome.

In all 14 cases of RA-PCP, corticosteroid was administered concomitantly with TMP-SMX. Two died, the mortality rate being 14%. High mortality has been reported in PCP of CTD (33% Sekowitz, 32% Godeau et al) (13, 22), to be much higher than AIDS-PCP. It is suggested that the good outcome of the present cases was the result of the use of corticosteroids added to TMP-SMX.

In AIDS-PCP, the National Institutes of Health - University of California Expert Panel recommends use of steroids as early as possible (27). In those cases, the inflammatory response evoked by *P. jirovecii* is assumed to contribute to the lung damage, indicating the need for corticosteroid treatment. However for RA-PCP or PCP of CTD in general, the validity of corticosteroid use has not been discussed in depth. Pareja et al retrospectively analyzed the clinical course of 30 cases of severe PCP without AIDS, among

whom 16 cases were treated with adjunctive corticosteroids (28). They reported good clinical outcome in patients who received high doses of adjunctive corticosteroids. In RA-PCP, the host inflammatory response is assumed to be more intense, in spite of lower organism burden, contributing to severe lung injury. It is therefore reasonable that corticosteroids may play a beneficial role in treatment of RA-PCP, when used concomitantly with antipneumocystic drugs. This issue should be examined in a prospective study.

Discrimination between RA-PCP and MTX-P

Comparison of clinical features of RA-PCP and MTX-P revealed their close resemblance, in terms of major symptoms, rapid progression, and severe oxygenation impairment. Levels of serum albumin, LDH, CRP, and KL-6 were also similar. Immunological status at presentation was also preserved relatively well in both groups. Thus, in the clinical setting of an acute respiratory event in a patient under MTX treatment for RA, discrimination between RA-PCP and MTX-P is challenging.

CT features have limited usefulness. When CT shows GGO of type A pattern or Type B pattern, MTX-P as well as RA-PCP are equally likely, because these patterns are seen in both diseases. Distinction is impossible by CT imaging alone. Thus the discrimination of RA-PCP from MTX-P should be based on detection of *P. jirovecii*, combined with serology.

In RA patients under MTX treatment with acute onset lung injury, we should treat them as MTX-P with corticosteroids, if *P. jirovecii* is not detected. If traditional staining or PCR reveals *P. jirovecii*, along with elevated plasma β-D-glucan level, we should treat it as RA-PCP, with antipneumocystic drugs. Use of adjunctive steroids is a matter to be examined in future.

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