医薬品
医薬部外品　研究報告 調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2004.8.26	該当なし	
一般的名称	乾燥濃縮人血液凝固第Ⅷ因子		研究報告の公表状況	N Engl J Med. 2004; 351(8):760-768.	公表国 米国	
販売名(企業名)	クロスエイトM250　(日本赤十字社) クロスエイトM500　(日本赤十字社) クロスエイトM1000　(日本赤十字社)					

研究報告の概要

ミニプール（16～24名の供血血液をプール）を実施した米国において核酸増幅検査を導入した最初の3年間は、この間にHIV-1 RNAもしくはHCV RNAが陽性であり抗体が陰性の全供血血液について解析した。検査した37,164,054単位のうち、12単位（供血310万例当たり1例）がHIV-1 RNA陽性と確認され、そのうちHIV-1 p24抗原が検出されたのは2例のみであった。HCVでは、39,721,404単位のうち170単位（供血23万例当たり1例）がHCV RNA陽性と確認された（より高感度のHCV抗体検査では27万例当たり1例）。核酸増幅検査による初回供血者のHCVおよびHIV-1の陽性率は、リピーターに比べそれぞれ3.3倍、4.1倍高かった。HCV RNA陽性供血者67名の追跡調査によると、セロコンバージョンは供血から中央値で35日後に起こり、その後ウイルス血症の消失する割合は低いことが立証された。長期間にわたり免疫学的に変化が認められなかったHCV感染も3例報告された。ミニプールの核酸増幅検査によって、年間約5件のHIV-1感染を、また約56件のHCV感染を防ぎ、輸血によるHIV-1感染、HCV感染の残存リスクは血液200万単位当たり約1単位に低下した。

報告企業の意見	今後の対応
米国において、ミニプール核酸増幅検査によって、年間HIV-1感染を約5件、HCV感染を約56件防ぐことが可能であり、輸血によるHIV-1感染、HCV感染の残余リスクを血液200万単位当たり約1単位に低下させたとの報告である。	これまで、本製剤によるHCV、HIV感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つ以上の異なるウイルス除去・不活化工程が含まれている。また最終製品についてHCV-NAT、HIV-NAT陰性であることを確認していることから、本製剤の安全性は確保されており、念のため情報収集に努めるも、今後特別の対応を必要としない。

使用上の注意記載状況・その他参考事項等

クロスエイトM250
クロスエイトM500
クロスエイトM1000
血液を原料とすることに由来する感染症伝播等
理論的な vCJD 等の伝播のリスク

ORIGINAL ARTICLE

Detection of HIV-1 and HCV Infections among Antibody-Negative Blood Donors by Nucleic Acid–Amplification Testing

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ABSTRACT

BACKGROUND

Testing of blood donors for human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) RNA by means of nucleic acid amplification was introduced in the United States as an investigational screening test in mid-1999 to identify donations made during the window period before seroconversion.

METHODS

We analyzed all antibody-nonreactive donations that were confirmed to be positive for HIV-1 and HCV RNA on nucleic acid–amplification testing of "minipools" (pools of 16 to 24 donations) by the main blood-collection programs in the United States during the first three years of nucleic acid screening.

RESULTS

Among 37,164,054 units screened, 12 were confirmed to be positive for HIV-1 RNA — or 1 in 3.1 million donations — only 2 of which were detected by HIV-1 p24 antigen testing. For HCV, of 39,721,404 units screened, 170 were confirmed to be positive for HCV RNA, or 1 in 230,000 donations (or 1 in 270,000 on the basis of 139 donations confirmed to be positive for HCV RNA with the use of a more sensitive HCV-antibody test). The respective rates of positive HCV and HIV-1 nucleic acid–amplification tests were 3.3 and 4.1 times as high among first-time donors as among donors who gave blood repeatedly. Follow-up studies of 67 HCV RNA–positive donors demonstrated that seroconversion occurred a median of 35 days after the index donation, followed by a low rate of resolution of viremia; three cases of long-term immunologically silent HCV infection were documented.

CONCLUSIONS

Minipool nucleic acid–amplification testing has helped prevent the transmission of approximately 5 HIV-1 infections and 56 HCV infections annually and has reduced the residual risk of transfusion-transmitted HIV-1 and HCV to approximately 1 in 2 million blood units.

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N Engl J Med 2004;351:760-8.

SCREENING OF POTENTIAL BLOOD DONORS has historically relied on the use of immunoassays to detect viral antibodies or antigens. In 1999, new screening methods involving nucleic acid amplification to detect human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) RNA were implemented in the United States under an investigational new drug protocol approved by the Food and Drug Administration (FDA).[1-3] This new technique was used to test multiple samples in small pools, referred to as "minipools." The decision to implement this technique was based on its ability to identify HIV-1– and HCV-infected donors early in the infectious window period, before seroconversion,[4] and the experience of plasma-derivative manufacturers showing the practicality of this approach for pooled specimens.[1] Finally, it was recognized that the availability of nucleic acid–based tests would support future testing of emerging agents.[5,6]

The advent of nucleic acid–amplification testing has led to the discontinuation of two less effective screening tests. HIV-1 p24 antigen screening was recommended by the FDA in 1996 for the early detection of HIV-1 infection,[7] and the FDA allowed this approach to be discontinued on the licensure of the HIV-1 nucleic acid–amplification test. Elevated levels of alanine aminotransferase have been used as a surrogate (nonspecific) marker for HCV infection since 1986.[8] The use of this screening approach was never an FDA requirement, so blood centers have voluntarily discontinued this test. RNA-based donor screening has afforded an opportunity to study events occurring early in HIV-1 and HCV infection.[9-12] To quantify the relative risk of transmission of HIV-1 and HCV from first-time blood donors and those who donated blood repeatedly, we analyzed the number of RNA-positive, antibody-nonreactive allogeneic blood donations from donors infected with HIV-1, HCV, or both that were identified in the first three years after the implementation of nucleic acid–amplification testing in the United States.

METHODS

Since 1999, allogeneic blood donations in the United States have been screened for HIV-1 and HCV RNA in a minipool format with the use of one of two nucleic acid–amplification tests.[1,2,13] The Gen-Probe Transcription-Mediated Amplification system uses a multiplex HIV-1 and HCV assay and minipools of 16 donor samples.[14] All donation samples within a reactive minipool are tested individually to identify both the sample that was reactive and the viral cause of the reaction. The Roche Molecular Systems Cobas AmpliScreen HIV-1 and HCV tests separately detect HIV-1 and HCV RNA in minipools of 24 donor samples.[11] Both assays are highly specific and sensitive, with 50 percent detection limits (i.e., the level at which 50 percent of test results would be expected to be reactive) of 14 or fewer copies of HIV-1 per milliliter and 12 or fewer copies of HCV per milliliter on the basis of probit analyses.[13,14] The 95 percent detection limits as defined in the package inserts for both tests range from 30 to 60 copies per milliliter for HIV-1 and HCV. Both systems have received FDA approval for routine screening of blood donors.

All major laboratories in the United States participating in nucleic acid–amplification screening (accounting for over 98 percent of tested blood donations) participated in this study and reported data collected on cases identified between March 1999 and January 2002, and in some instances from March 1999 through April 2002. A case was defined as an allogeneic donation that was nonreactive to antibody against HIV-1, HCV, or both but that was reactive on minipool nucleic acid–amplification screening and confirmed to be positive for HIV-1 or HCV RNA. Five testing programs used the Gen-Probe assay and reported cases of HIV-1 and HCV viremia identified on screening of 27,956,758 donations. The Roche Cobas AmpliScreen was used in 13 laboratories, which tested a total of 9,207,296 donations for HIV-1 RNA and 11,764,646 donations for HCV RNA. All participating sites received approval of this study from their institutional review board. Data were contributed by the blood-collection organizations and the manufacturers of the nucleic acid assays (Roche Molecular Systems, Gen-Probe, and Chiron).

The date of donation, the donor's status as a first-time or repeat donor, and whether the unit would have qualified for transfusion if not for the result of the nucleic acid–amplification test (i.e., whether the unit was transfusable) were collected for each case. Furthermore, the results of HIV-1 p24 antigen testing were compiled for cases of HIV-1 viremia, whereas data on alanine aminotransferase levels and the presence or absence of antibody against hepatitis B core antigen (anti-HBc) were col-

lected for cases of HCV viremia. When applicable, we also compiled the results of repeated serologic analyses, repeated nucleic acid–amplification testing of the index sample with the use of a different type of RNA method (e.g., different techniques, primers, or probes), nucleic acid–amplification testing of an independent sample from the index donation, and serologic and nucleic acid–amplification testing of samples collected from donors participating in the follow-up analysis. For HIV-1, antibody was detected with the use of enzyme immunoassays and confirmed by Western blotting; for HCV, antibodies were detected by either second- or third-generation enzyme immunoassays and confirmed by recombinant immunoblot assay (RIBA, Chiron). Laboratories that routinely used second-generation HCV-antibody tests to screen donations were also asked to report the results of third-generation HCV-antibody tests performed on the HCV RNA–positive donations. This allowed categorization of cases of HCV viremia into those in which antibodies were detectable only by the more sensitive third-generation test and those with no detectable HCV antibody on both second- and third-generation HCV-antibody tests.[11] A case was considered confirmed if the index donation was reactive to HIV-1 or HCV RNA with the use of a second type of nucleic acid–amplification test, if another sample from the index donation was reactive on the nucleic acid assay, or if at least one follow-up sample was reactive on nucleic acid–amplification testing or antibody testing.

An expanded data set was developed by the largest participating program (the American Red Cross) to study the dynamics of HCV infection. This data set included follow-up of HCV RNA–positive donors identified from March 1999 through mid-June 2003, thus providing an additional 15 months of follow-up on a well-characterized group of donors with acute HCV infection. For this program, a standardized prospective protocol was used to enroll donors, with specimens collected at approximately four-week intervals through the time of seroconversion, as confirmed by third-generation HCV-antibody tests, and beyond.

To determine whether trends observed for HIV-1–positive and HCV RNA–positive donors were constant beyond this three-year study, an additional two years of data from the American Red Cross were analyzed. Data on HIV-1–positive and HCV RNA–positive donors from March 1999 through March 2002 were compared with those for the subsequent two-year period from April 2002 to April 2004.

To evaluate rates of positive nucleic acid–amplification tests for HIV-1 and HCV RNA in specific subgroups (first-time and repeat donors and donors with otherwise transfusable donations), data were included only from laboratories that routinely reported this information. These subgroups represented about 37.0 million of the 39.7 million total donations. On the basis of data from the American Red Cross for 1999 through 2002, it was estimated that 23 percent of allogeneic donations were collected from first-time donors and 1 percent of all donations were discarded owing to reactivity to another routine serologic screening test in addition to nucleic acid–amplification testing.[15]

Rates of positive nucleic acid–amplification tests per million donations were calculated by dividing the number of cases by the number of known donations screened (or for samples from first-time or repeat donors and samples that were otherwise transfusable, by the estimated number of donations) and multiplying by 10^6. When the number of donations was known, the associated 95 percent confidence interval for the rate was computed.[16] When the number in a subgroup of donations was estimated, an approximate 95 percent confidence interval was computed incorporating the uncertainty around the estimated number of donations.[16,17] Fisher's exact tests and Wilcoxon's tests were used to compare categorical and continuous variables, respectively. All reported P values are two-sided.

RESULTS

VIREMIC, SERONEGATIVE DONATIONS DETECTED BY NUCLEIC ACID–AMPLIFICATION TESTING

In the three years after the implementation of minipool nucleic acid–amplification testing, 12 donations that were not reactive to HIV-1 antibody and 170 donations that were not reactive to HCV antibody were confirmed to be positive for HIV-1 RNA and HCV RNA, respectively, among approximately 37 million to 40 million donations screened (Table 1). Hence, 1 per 3.1 million donations screened was confirmed to be positive for HIV-1 RNA and antibody-nonreactive, whereas 1 donation per 230,000 was confirmed to be positive for HCV RNA and antibody-nonreactive. Rates did not differ significantly between users of the Gen-Probe nucleic acid–amplification test and users of the Roche test (P=0.74, data not shown).

EFFECT OF SEROLOGIC SCREENING ASSAYS ON THE DETECTION OF HCV RNA

In the United States, laboratories use one of two licensed assays, which differ significantly in window-period sensitivity, to screen donations for HCV antibody.[11,18] Of 156 HCV RNA–positive donations, 17 that were antibody-nonreactive on the second-generation assay would have been identified as reactive by the third-generation assay, adjusting the rate of HCV-positive donations to 1 in 270,000 donations (Table 1).

Some of the donations that were positive on minipool nucleic acid–amplification testing would not have been released for transfusion even if such testing had not been performed. Among the 12 donations that were positive for HIV-1 RNA, 2 were confirmed to be positive for HIV-1 p24 antigen (Table 1), and 33 percent of donations that were identified as positive for HCV RNA (51 of 156) by laboratories that reported subgroup information would have been deemed nontransfusable (Table 1), including 45 of 51 units (88 percent) with an elevated alanine aminotransferase level. The remaining 6 were nontransfusable owing to reactivity to other routine screening tests; none of 155 HCV RNA–positive donations evaluated for anti-HBc reactivity were reactive. Thus, HCV nucleic acid screening prevented the release of 1 viremic donation for every 350,000 donations screened.

Table 1. Rates of Positivity for HIV-1 and HCV RNA, March 1999 to April 2002.

	No. of Donations	RNA-Positive Donations	
		Total No.	Rate per 10^6 Donations (95% CI)*
HIV-1			
Total	37,164,054	12	0.32 (0.17–0.56)
Otherwise transfusable	36,792,000†	10	0.27 (0.13–0.50)
HCV			
Total	39,721,404	170	4.3 (3.7–5.0)
Subtotal‡	36,974,722	156	4.2 (3.6–4.9)
Third-generation antibody–nonreactive‡	36,974,722	139	3.8 (3.2–4.4)
Otherwise transfusable†	36,605,000†	105§	2.9 (2.3–3.5)

* CI denotes confidence interval.
† Ninety-nine percent of donations were assumed to be transfusable (nonreactive on all screening tests and suitable for transfusion).
‡ Data from three laboratories were excluded because they did not report data on transfusability according to donors' first-time or repeat status, or the results of third-generation assays for the RNA-positive index donations.
§ One HCV RNA–positive donation with missing data on transfusability was weighted according to the distribution of transfusable and nontransfusable units among the remaining HCV RNA–positive donations, so that 104.67 (rounded to 105) such donations were assumed to be otherwise transfusable.

ALANINE AMINOTRANSFERASE PATTERNS IN DIFFERENT STAGES OF HCV INFECTION

To compare the distribution of alanine aminotransferase levels associated with various stages of HCV infection, we evaluated donor alanine aminotransferase levels compiled by the American Red Cross from 1999 through 2002. As shown in Figure 1, HCV-seronegative donors had significantly different alanine aminotransferase distributions depending on their HCV RNA status; donors confirmed to be positive for HCV RNA had higher median enzyme levels than HCV RNA–negative donors (54 vs. 21 IU per liter, $P<0.001$). Donors who were confirmed to be positive for HCV RNA had elevated enzyme levels independent of their HCV antibody status (median, 56 IU per liter for seropositive donors vs. 54 IU per liter for seronegative donors; $P=0.99$). However, enzyme elevations of 120 IU per liter or more were noted more frequently among HCV RNA–positive, seronegative donors than among HCV RNA–positive, seropositive donors (30 percent vs. 15 percent, $P<0.001$). Lastly, among seropositive donors, enzyme levels were again higher among HCV RNA–positive donors than among HCV RNA–negative donors (56 vs. 22 IU per liter, $P<0.001$).

RELATIVE YIELD OF NUCLEIC ACID–AMPLIFICATION TESTS AMONG FIRST-TIME DONORS AND REPEAT DONORS

Viremic donations were more likely to be detected from first-time rather than repeat donors. Although only marginally significant ($P=0.05$), the rate of positivity for HIV-1 RNA was 4.1 times as high among the former group as the latter group; this ratio was 2.7 for the donations that were nonreactive to HIV-1 p24 antigen but positive for HIV-1 RNA (Table 2). The rate of positivity for HCV RNA was 3.3 times as high among first-time donors as among repeat donors ($P<0.001$) (Table 2); the rate among first-time donors was similarly elevated when the calculations were restricted to HCV RNA–positive donations that were nonreactive on third-generation assays (7.9 per 10^6 units from first-time donors vs. 2.5 per 10^6 units from repeat donors; rate ratio, 3.2; 95 percent confidence interval, 2.0 to 5.0).

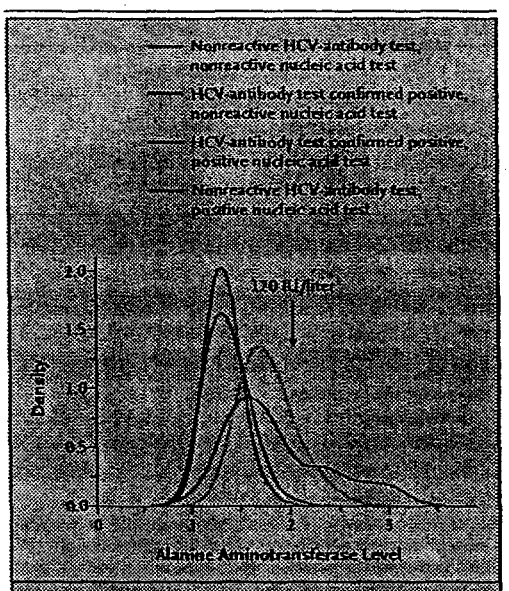

Figure 1. Distribution of Alanine Aminotransferase Levels According to HCV Status.

The alanine aminotransferase values, which are given on a base 10 logarithmic scale, in each of the four groups were used to estimate probability densities.

FOLLOW-UP INVESTIGATIONS OF RNA-POSITIVE DONORS

Follow-up studies of seronegative donors who were confirmed to be positive for viral RNA demonstrated that these donations were virtually all made in the early stage of infection when viremia is present but an antibody reaction cannot be detected. Eight of 12 donors with positive HIV-1 nucleic acid–amplification tests enrolled in follow-up. All eight seroconverted within six weeks after the positive test. The median interval between the RNA-positive index donation and the first antibody-reactive sample was 11.5 days (range, 6 to 42), and the median interval between the donation and the first confirmed seropositive sample was 20.5 days (range, 15 to 42). These intervals probably represent an overestimate of the actual time to seroconversion, since the interval between follow-up samples varied and the sample size was small. Data were available for six additional HIV-1 RNA–positive donors (identified from April 2002 through April 2004) and demonstrated a similar time to seroconversion (8.6 days to antibody reactivity and 20.5 days to confirmed positivity).

For HCV, 90 of the 139 HCV RNA–positive donors who had nonreactive third-generation assays

Table 2. Rates of Positivity for HIV-1 and HCV RNA among First-Time and Repeat Donors, March 1999 to April 2002.*

Donor Status	Total No. of Donations	Total No.	RNA-Positive Donations	
			Rate per 10^6 Donations (95% CI)	Ratio of First-Time Donors to Repeat Donors (95% CI)
HIV-1†				
First-time	8,178,000‡	6	0.73 (0.24–1.81)	4.1 (1.0–17.0)§
Repeat	27,692,000‡	5	0.18 (0.06–0.43)	
HCV¶				
First-time	8,430,000‡	77	9.1 (6.4–12.9)	3.3 (2.1–5.1)
Repeat	28,544,000‡	79	2.8 (2.1–3.6)	

* CI denotes confidence interval.

† Data from one laboratory were excluded because the laboratory did not report donors' status (first-time or repeat).

‡ A total of 22.8 percent of donations were assumed to be from first-time donors and 77.2 percent from repeat donors.[15]

§ If calculations were restricted to RNA-positive donations that were nonreactive to HIV-1 p24 antigen, the rates were 0.49 per million first-time donors (95 percent confidence interval, 0.12 to 1.41) and 0.18 per million repeat donors (95 percent confidence interval, 0.06 to 0.43), resulting in a ratio of 2.7 (95 percent confidence interval, 0.5 to 12.7).

¶ Data from three laboratories were excluded because they did not report donors' status, data on transfusability, or the results of HCV third-generation assays for the HCV RNA–positive index donations. Five HCV RNA–positive donations with missing data on donor status were weighted according to the distribution of first-time and repeat donors among the remaining HCV RNA–positive donations.

enrolled in the follow-up study; 75 of the 90 seroconverted. In the majority of those who did not seroconvert, the duration of follow-up was too short (range, 12 to 58 days) to allow determinations of their eventual seroconversion status.

The expanded data set from the American Red Cross allowed more extensive evaluation of the dynamics of HCV seroconversion. Figure 2 provides the follow-up results for 67 HCV RNA–positive donors (48 identified from March 1999 through April 2002, plus 19 identified from May 2002 through June 2003). Of these, 7 (10 percent) discontinued follow-up before either seroconversion or three months of follow-up, the interval during which seroconversion generally occurred (Fig. 2A), and 55 (82 percent) seroconverted. The median time to seroconversion (from the RNA-positive, antibody-nonreactive index donation to a reactive third-generation antibody test) was 35 days. This is likely to be an underestimate of the viremic, antibody-nonreactive window period, since the period of viremia before the index donation is unknown. Of the 55 donors who seroconverted, 47 remained viremic during continued follow-up; 3 donors had fluctuating viremia in the presence of HCV antibody, and in 5, the HCV infection resolved after seroconver-

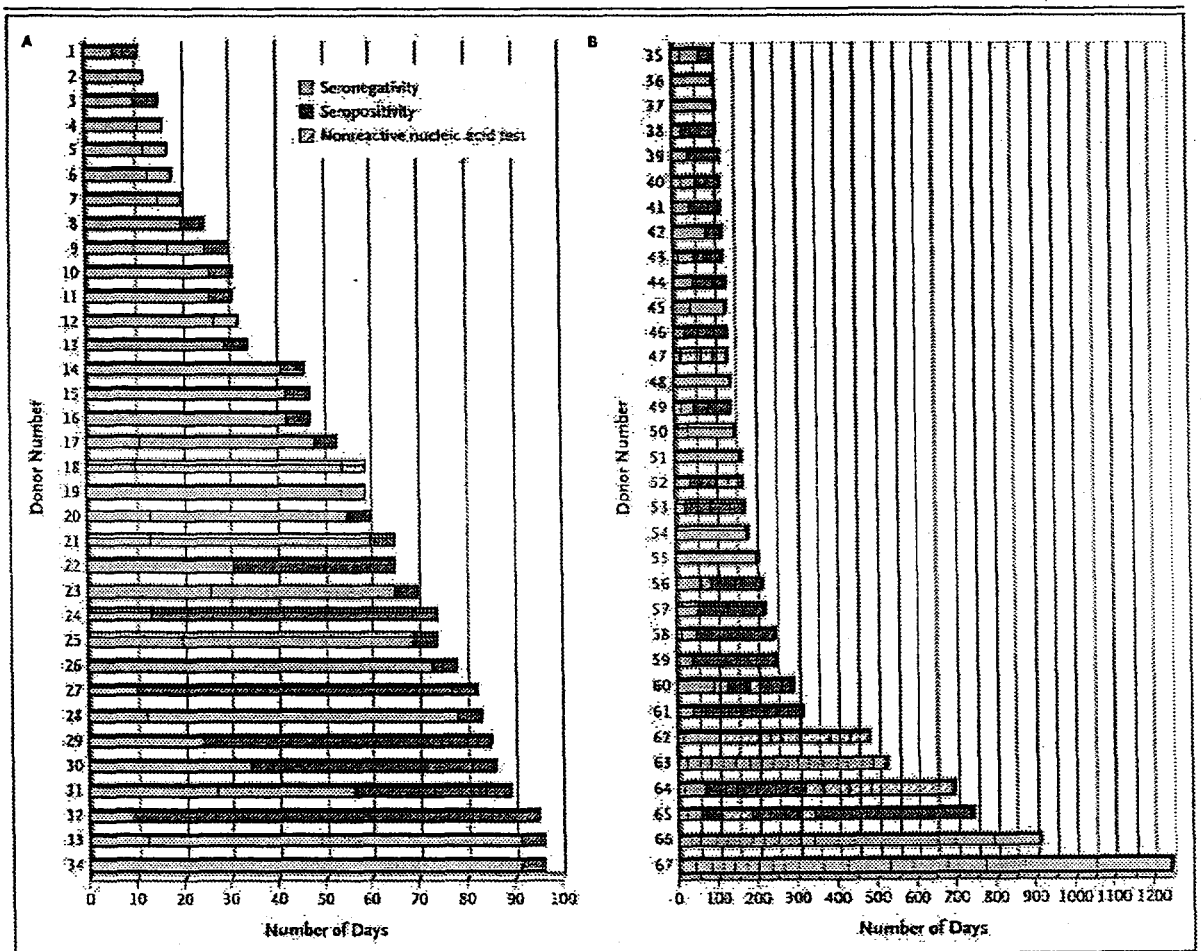

Figure 2. Follow-up Results for 67 HCV RNA–Positive, HCV Antibody–Nonreactive Donors.

Data were provided by the American Red Cross. Panel A shows the results for those with no more than 92 days of follow-up, and Panel B the results for those with more than 92 days of follow-up. A red tip at the end of a yellow line indicates that the donor was seropositive on the last day of follow-up.

sion, with persistent RNA negativity for up to one year (Fig. 2B). Two additional donors (3 percent) had an abortive HCV infection, in which HCV RNA could initially be repeatedly demonstrated shortly after enrollment but disappeared in the absence of HCV seroconversion. Lastly, three donors (4 percent) remained viremic without elevated alanine aminotransferase levels, but they did not seroconvert after a follow-up period ranging from 1.5 to more than 3 years (so-called immunologically silent infections) (Fig. 2B). The donor with the longest immunologically silent period was infectious during this period, since his RNA-positive donation transmitted HCV to a platelet recipient early in the nucleic acid–amplification testing program before the American Red Cross began withholding all blood components until the results of such tests were available.[12]

HIV-1–POSITIVE AND HCV-POSITIVE DONORS IDENTIFIED FROM APRIL 2002 TO APRIL 2004

An additional two years of data from the American Red Cross demonstrated no changes in the rates of positivity for HCV RNA — from 1 in 251,000 for the period from March 1999 through March 2002 (79 per 19,811,809 donations screened) to 1 in 222,200 for the period from April 2002 through April 2004 (60 per 13,332,257 donations screened, P=0.49). For HIV-1, the rates were 1 in 4 million and 1 in 2.2 million, respectively. A similar number of HIV-1 RNA–positive donations were identified during the two periods (five and six, respectively). Even though the frequency of HIV-1 RNA–positive donations increased for the period from April 2002 through April 2004, this increase was not significant (P=0.37).

DISCUSSION

Assuming that each of the 13.6 million allogeneic units of blood donated annually in the United States is converted on average to 1.45 transfusable components,[19,20] our data indicate that the implementation of minipool nucleic acid screening likely prevented about 5 cases of transfusion-transmitted HIV-1 infection and 56 cases of HCV infection annually. The documented findings are consistent with the those predicted from mathematical models.[4,15,21] Despite the fact that these rates are relatively low and have remained stable for five years, implementation of these tests was consistent with the goal of maximizing blood safety.[1,3] It has been estimated that nucleic acid screening has reduced the residual risk of transfusion-associated infection for both HIV-1 and HCV to about 1 in 2 million blood units from repeated donors.[15] This is a reduction from rates of 1 in 276,000 for HCV and 1 in 1.5 million for HIV-1 with the use of serologic testing alone.[15] The residual risk after the implementation of nucleic acid–amplification testing results from the presence of virus below the limit of detection of minipool testing[22]; individual nucleic acid screening of each sample, rather than screening of small pools of multiple samples, would further decrease the residual risk but at a substantially greater cost.

With the licensure of nucleic acid–amplification tests, the FDA has permitted the discontinuation of HIV-1 p24 antigen testing on the basis of data showing that HIV-1 RNA screening is better able to detect infection in the window period shortly after infection and that all p24 antigen–positive donations are also RNA-positive.[7,23] This policy is supported by our data, in which HIV-1 nucleic acid screening identified 12 infected donors, only 2 of whom were identified by p24 antigen testing; in contrast, there were no RNA-negative donations from HIV-1–infected donors that were identified as positive by p24 antigen testing. The detection of p24 antigen in the absence of antibody corresponds to the peak viremic period when blood donors are likely to defer donations owing to influenza-like symptoms.[7,9,24]

Approximately one third of the units detected by HCV nucleic acid–amplification testing would have been discarded anyway owing to elevated alanine aminotransferase levels. Because of the relative nonspecificity of this surrogate marker, the absence of evidence of additional transfusion-transmissible hepatitis agents,[8,25] and the implementation of a sensitive screening method for the detection of HCV RNA, the continued use of alanine aminotransferase screening for preventing transfusion-associated hepatitis is no longer justified; consequently, many blood centers have stopped using this test. In addition, the presence of circulating HCV RNA is a direct marker of viral replication and indicates a diagnosis of HCV infection with greater sensitivity and specificity than does the presence of elevated liver enzymes.

Our data show that new HCV and HIV-1 infections occur three to four times as often among first-time donors as among repeat donors, substantiating previous observations.[15,26] This finding sup-

ports the general principle that retention of repeat donors enhances both the adequacy and safety of the blood supply. Possible reasons for higher rates among first-time donors include inappropriate use of blood donation to obtain the results of viral tests; failure to understand the questions for donors and, hence, the donor-selection criteria; and self-deferral of the donor after the first donation owing to the realization that his or her donation was unsuitable.

The routine use of nucleic acid–amplification tests and serologic assays for donor screening has made possible the identification of persons in the very early stages of HIV-1 and HCV infection; this information can provide insights into risk factors associated with viral infection and potentially contribute to studies of the natural history, pathogenesis, and treatment of these infections.[9,10] For example, an analysis of recent risk-related behavior among HCV-infected donors identified by nucleic acid–amplification testing may identify behavioral and demographic characteristics that could be used to improve donor-qualification criteria, provided effective questions could be designed.[27] The addition of HCV RNA testing to routine HCV-antibody screening has also allowed seropositive donors to be subdivided into those with active infection (plasma RNA–positive) and those with either resolved HCV infection or intermittent viremia (plasma RNA–negative at the time of donation). Enrollment of these donors into natural-history and early-treatment trials could enhance our understanding of the pathogenesis of HCV infection, including the factors underlying the spontaneous resolution of HCV viremia.[10,28]

Several reports have suggested that serologic testing may miss a substantial proportion of infected persons.[29-31] We found that only three seronegative donors with persistent hepatitis C viremia did not seroconvert during the expected time frame. During this same time at the American Red Cross, more than 800 HIV-seropositive donors and more than 16,000 HCV-seropositive donors were identified. Thus, persistent immunologically silent infections are extremely rare, reinforcing the continued reliance on serologic analyses for HIV-1 and HCV as the primary tools for diagnostic testing.[32]

Because blood centers had already implemented nucleic acid–amplification testing for HIV-1 and HCV, it was feasible in 2003, in collaboration with the Centers for Disease Control and Prevention and the FDA and with the rapid development of nucleic acid–amplification tests by manufacturers, to implement screening for West Nile virus in less than nine months.[33-35] Results indicate that close to 1000 donors with West Nile virus infection were identified by nucleic acid–amplification testing in 2003 and their donations discarded, probably preventing more than 1000 transfusion-related infections.[35]

The relatively low yield and poor cost effectiveness of HIV-1 and HCV minipool nucleic acid–amplification testing have led some to question the value of such screening. Using somewhat different analyses and assumptions, two independent groups studying the cost-effectiveness of HIV-1 and HCV minipool nucleic acid–amplification testing, both in the context of eliminating p24 antigen screening, estimated costs of $1.5 million to $4.3 million per quality-adjusted year of life.[19,36] Costs increase further if each donated blood unit is to be tested rather than combined in minipools, with yet further increases in cost for the automation required to perform large numbers of individual screening tests. Therefore, the cost of HIV-1 and HCV nucleic acid–amplification testing would need to decrease substantially to bring it in line with that of most other accepted medical practices. However, the aggregate cost-effectiveness of nucleic acid–amplification testing may have substantially improved with the implementation of such screening for West Nile virus. The rapid development and introduction of nucleic acid screening for West Nile virus and the ability to expand nucleic acid–amplification testing to include other emerging infections in the future further serve to support the adoption of this important tool for the screening of blood donations.

Supported by the individual blood programs represented as well as by contracts (N01-HB-97077 [superseded by N01-HB-47114], N01-HB-97078, N01-HB-97079, N01-HB-97080, N01-HB-97081, and N01-HB-97082) with the National Heart, Lung, and Blood Institute.

Dr. Busch reports having received consulting or lecture fees from Abbott Diagnostics, Acrometrix, Haemonetics, Navigant/Gambro, and Ortho-Clinical Diagnostics; Dr. Dodd consulting or lecture fees from Chiron and Roche Biomedical; Dr. Kleinman consulting fees from Chiron and Roche Molecular Systems; Dr. Stramer consulting fees from Chiron and Gen-Probe; and Dr. Strong consulting or lecture fees from Roche Molecular Systems. Dr. Strong also reports owning equity in Human BioSystems.

We are indebted to S. Matthew, R. McEntire, A. Snowhite, D. Todd, and Y. Xu for data collection and programming at Westat; and to E. Notari, A. Wagner, J. Paolillo, M. Beyers, M. Parcells, and K. Kane for data management and analysis of the surveillance and follow-up study.

APPENDIX

The National Heart, Lung, and Blood Institute Nucleic Acid Test Study involves the following sites and investigators: National Heart, Lung, and Blood Institute, National Institutes of Health — G. Nemo; Blood Centers of the Pacific and Blood Systems — M. Busch (principal investigator); Westat — G. Schreiber, M. King, S. Kleinman, S. Glynn; American Red Cross (Gen-Probe site) — S. Stramer, R. Dodd, J. Brodsky, J. Davis; America's Blood Centers (Gen-Probe sites: Blood Center of Southeastern Wisconsin, Blood Systems Laboratories, and Florida Blood Services, and Roche sites: Blood Center of Southeast Louisiana, BloodSource, Bonfils Blood Center, Central Florida Blood Bank, Community Blood Center of Greater Kansas City, Gulf Coast Regional Blood Center, LifeSource Blood Services, LifeSouth Community Blood Centers, Memorial Blood Centers of Minneapolis, New York Blood Center, Oklahoma Blood Institute, and Puget Sound Blood Center) — S. Caglioti, D.M. Strong; Association of Independent Blood Centers (Gen-Probe site) — R. Gammon; Center for Biologics Evaluation and Research, FDA — L. Hewlett; Roche — J. Gallarda, Y. Yang; Gen-Probe — L. Mimms, C. Giachetti, S. McDonough; Chiron — B. Phelps; Stanford Medical School blood bank.

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医薬品
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識別番号・報告回数			報告日	第一報入手日 2004 年 4 月 27 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②③④人血清アルブミン ⑤乾燥濃縮人血液凝固第Ⅷ因子 ⑥乾燥濃縮人血液凝固第Ⅸ因子	研究報告の公表状況	第 73 回日本寄生虫学会大会/ Ⅱ-C-37	公表国 日本		
販売名 （企業名）	①献血アルブミン-Wf（ベネシス） ②献血アルブミン(5%)-Wf（ベネシス） ③アルブミン-Wf（ベネシス） ④アルブミン-ヨシトミ(20%)（ベネシス） ⑤コンコエイトーHT（ベネシス） ⑥クリスマシンーM（ベネシス）					

研究報告の概要

近年、日本の各地でラテンアメリカからの日系労働者が定着し、その数は 33 万人を超えようとしている。ブラジルからは約 26 万人、ペルーから約 5 万人、続いてアルゼンチン、ボリビア、パラグアイとなっている。南米の風土病のひとつに治療法が確立していない Chagas 病がある。最近 5 年間に当教室において、その疑いもしくは確定診断の目的で 14 例、平均年齢（42.3 歳）の血清免疫診断を行った。その結果、11 例（78%）が病原体 Trypanosoma cruzi（T. cruzi）に対する IgG 抗体が陽性であり、中には高い IgG 抗体価（4,096 倍以上）を示し、PCR 法にて T. cruzi-DNA を検出した例もあった。国別ではブラジル 9 例、ボリビア 2 例であり、その共通点は、①全例重篤な心筋伝達障害での緊急入院、②居住歴に本疾患の主媒介昆虫である Triatoma infestants（T. infestants）が生息していた地域もしくは現在も生息している地域がある。また、輸血による感染を示唆する 24 歳の症例も経験した。彼らは自覚症状がないまま来日し、就労生活を続け、突然の発症発作に見舞われたが、臨床的にはすでに感染慢性期の状態であった。本疾患の末期症状の発現までには無症状の期間が長く、感染者本人、家族も無関心ですごすことが多いが、彼らは Chagas 病のキャリアである。

使用上の注意記載状況・その他参考事項等

代表として献血アルブミン-Wf の記載を示す。

2. 重要な基本的注意

(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している.更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アルブミンを精製し、アルブミン濃度 5w/v%に調整した製剤であり、ウイルス不活化を目的として、製造工程において 60℃、10 時間の液状加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
ラテンアメリカから定着した日系労働者の中に Chagas 病のキャリアがいるとの報告である。Chagas 病の病原体である Trypanosoma cruzi の大きさは 2～3μm である。万一、本剤の原料血漿に混入したとしても 0.22μm の除菌ろ過等の製造工程にて不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

II-C-37

日系ラテンアメリカ人の国内発症Chagas病とその背景:三浦左千夫[1]、北村　憲一[2]、白倉　哲郎[3]、竹内　勤[1]([1]慶大・医・熱医寄生虫学、[2]滋賀医大・内科、[3]昭和医大・医動物学)

Chagas' dease and its' background for descendents of Japanese migrants workers from Latin America.([1]Dept.Med.Trop & Parasitol.,KeioUniv.Sch.Med.[2]Dept.Med.,Div.Hem.,ShigaUniv.Med Sci.,[3]Dept.Med Zool.,Showa Sch.Med)

近年、日本の各地でラテンアメリカからの日系就労者が定着し、その数は３３万人を超えようとしている。ブラジルからは約２６万、ペルーから約５万人、続いてアルゼンチン、ボリビア、パラグァイとなっている。南米の風土病のひとつに治療法が確立されていないChagas病がある。最近５年間に当教室において、その疑いもしくは確定診断の目的で１４例、平均年齢(４２．３歳)の血清免疫診断を行った。その結果１１例（７８%）が病原体 *Trypanosoma cruzi* (*T. cruzi*) に対するIgG抗体が陽性であり、中には高いIgG抗体価（４０９６倍以上）を示し、PCR法にて *T. cruzi*-DNA を検出した例もあった。国別ではブラジル９例、ボリビア２例であり、その共通点は、①全例重篤な心筋伝導障害での救急入院、②居住歴に本疾患の主媒介昆虫である *Triatoma infestans*(*T. infestans*)が生息していた地域もしくは現在も生息している地域がある。また輸血による感染を示唆する２４歳の症例も経験した。彼らは自覚症状がないまま来日し、就労生活を続け、突然の発症発作に見舞われたが、臨床的にはすでに感染慢性期の状態であった。本疾患の末期症状の発現までには無症状の時期が長く、感染者本人、家族も無関心ですごすことが多いが、彼らは Chagas 病のキャリアーである。

II-C-38

Babesia gibsoni 感染症の分子疫学調査および loop-mediated isothermal amplification (LAMP)法を用いた診断法の有用性　筏井宏実[1]、田中浩子[1]、柴原乃奈[1]、松鵜　彩[2]、工藤　上[1]、五十嵐郁男[3]、小山田　隆[1]([1]北里大・獣医・寄生虫学、[2]北里大・獣医・小動物臨床学、[3]帯広畜産大・原虫研)

Molecular evidence of *B. gibsoni* parasite infections in Japan and evaluation of the diagnostic potential of a LAMP method. Hiromi Ikadai[1], Hiroko Tanaka[1], Nona Shibahara[1], Aya Matsuu[2], Noboru Kudo[1], Ikuo Igarashi[4], Takashi Oyamada[1] ([1]Dept. Vet. Parasitol. & [2]Small Animal Med., Kitasato Univ., [4]Nat. Res. Cent. Protozoan Dis., Obihiro Univ.)

犬 *B. gibsoni* 感染症は、主に西日本での発生が多く報告されており、東日本における感染状況については不明な点が多い。本研究では PCR 法を用いて青森県および沖縄県における *B. gibsoni* 感染症の調査を行なった。さらに、新しい遺伝子増幅法である LAMP 法を用いた診断法の有用性についても検討を行った。

2002 年 7 月から 2003 年 7 月にかけて青森県および沖縄県の小動物病院に来院した 1,082 頭の犬血液サンプルについて調査した結果、青森県 945 頭中 37 頭および沖縄県 137 頭中 15 頭が PCR 法で陽性を示した。青森県で認められた陽性犬は全て土佐犬の雄であった。沖縄県では陽性犬の犬種、性別および年齢は様々であった。青森県と沖縄県で確認された本原虫の 18S rDNA 塩基配列を決定した結果、全てのサンプルにおいて報告されている配列と100%一致した。さらに 18S rDNA を基に LAMP プライマーを設計し、赤血球内寄生率 5.0%の感染犬血液を用いた階段希釈法により PCR 法と LAMP 法の検出感度を比較した結果、両方法とも検出限界は 0.0005%であり、LAMP 法は PCR 法と同様に感度が高いことが確認された。また、LAMP 法を用いて沖縄県の犬 137 頭分の血液サンプルを調べた結果、137 頭中 15 頭が陽性を示し、PCR 法を用いて調査を行なった結果と一致した。

以上より、青森県における *B. gibsoni* 感染症は土佐犬のみに浸潤している事が明らかとなった。また、LAMP 法を用いた本感染症の診断は、高感度で簡易かつ迅速な診断法として有用な手法であることが明らかとなった。　(平成 14-16 年度　若手 B)

II-C-39

Multiplex PCR のヒト・テニア症分子疫学への応用

山崎 浩[1]、James C. Allan[2]、Philip S. Craig[3]、Toni Wandra[4]、Thomas Suroso[4]、中尾 稔[1]、迫 康仁[1]、Wulamu Mamuti[1]、中谷和宏[5]、伊藤亮[1]([1]旭川医大・寄生虫、[5]同・実験動物、[2]ファイザー株、[3]サルフォード大、[4]インドネシア厚生省)

Application of multiplex PCR to molecular epidemiology of taeniasis in humans. H. Yamasaki[1], JC Allan[2], PS Craig[3], T Wandra[4], T Suroso[4], M Nakao[1], Y Sako[1], W Mamuti[1], K Nakaya[5], A. Ito[1] ([1]Dept. Parasitol., [5]Animal Lab., Asahikawa Med. Col., [2]Pfizer Ltd., [3]Univ. Salford, [4]Ministry Health, Indonesia)

ヒト・テニア症は *Taenia solium*（有鉤条虫）、*T. saginata*（無鉤条虫）、および *T. asiatica* (仮称、アジア条虫)の成虫寄生によって惹き起こされるが、臨床症状は一般に軽微である。しかしながら、条虫キャリアから排泄される成熟受胎片節は中間宿主にとって新たな感染源となり、特に有鉤条虫キャリアの場合、キャリアの早期発見と治療は有鉤嚢虫症対策上きわめて重要である。演者らは条虫キャリア検出を目的とした multiplex PCR 法の有用性を昨年の本学会で報告した。今回、中米・グアテマラの有鉤条虫キャリア (n=14)、ならびにインドネシア・バリ島の無鉤条虫キャリア (n=11)の糞便を用いて multiplex PCR のテニア症分子疫学への有用性を検討した。**[材料と方法]** 供試した 25 症例は駆虫によって排泄された片節の形態、あるいはそのミトコンドリア遺伝子検査、さらに有鉤条虫キャリアについては糞便内抗原検査によっても確定診断されたものである。糞便内DNA調製と cytochrome c oxidase subunit 1 遺伝子(*cox1*)を標的にした multiplex PCR は Yamasaki et al. (2003)に準じて行った。**[結果と結論]** Multiplex PCR によって、グアテマラの有鉤条虫キャリアでは 14 検体中 7 例で 720 bp の *cox1* 断片が検出され、残り 7 例では検出されなかった。そこで、*cox1* 全長をまず増幅し、これを鋳型にして有鉤条虫 American genotype 特異的プライマーを用いた nested PCR を行ったところ、7 例中 5 例で *cox1* 断片が検出された。虫卵排出のない未熟片節キャリアにおいても *cox1* 断片が検出されたことは、PCR 検査によってキャリアの早期発見が可能であることを示唆している。一方、インドネシアの無鉤条虫キャリアでは 827 bp の *cox1* 断片が 11 例全例から検出された。以上の結果から、multiplex PCR は種特異的で感度の高い検査法としてテニア症流行地における分子疫学的研究に有用であることが示された。

(平成14-17年度　基盤A海外)

Yamasaki et al. 2003.　J. Clin. Microbiol. (in press).

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ウエスタンブロット法によるエクアドルの皮膚リーシュマニア症の血清診断と抗原分析　尾池貴洋[1]、牛久妙[1]、Calvopina M.[2]、Marco J. D.[2]、Barroso P. A.[2]、撹口義久[2]、加藤大智[2]、Gomez E.[2]、上里博[3]、野中薫雄[3]、三森龍之[4]、鈴木守[1]、片倉賢[1]([1]群馬大・院医・国際寄生虫病生態学、[2]高知大・医・寄生虫学、[3]山口大・農・獣医家畜衛生学、[4]カトリカ大・エクアドル、[5]琉球大・医・皮膚科、[6]熊本大・院医薬・腫瘍)

Serodiagnosis of cutaneous leishmaniasis in Ecuador and evaluation of *Leishmania* antigens by Western blot. Takahiro Oike[1], Tae Ushiku[1], Manuel Calvopina[2], Jorge D. Marco[2], Paola A. Barroso[2], Yoshihisa Hashiguchi[2], Hirotomo Kato[2], Eduardo Gomez[2], Hiroshi Uezato[2], Shigeo Nonaka[3], Tatsuyuki Mimori[3], Mamoru Suzuki[3], Ken Katakura[3] ([1]Dept. Parasitol., Gunma Univ. Gradu. Sch. Med. Sci., [2]Dept. Parasitol., Kochi Med. Sch., [3]Dept. Vet. Hyg., Fac. Agr., Yamaguchi Univ., [4]Catolica Univ., Ecuador, [5]Dept. Dermatol., Ryukyu Univ., [6]Dept. Genetic Tumor & Bio., Gradu. Sch. Med. Sci. Kumamoto Univ.)

皮膚リーシュマニア症の診断には、病変部生検材料からの原虫の検出や PCR による原虫 DNA の検出が行われる。しかし、治癒傾向にある皮膚病変部からの生検材料採取は困難であり、また他の皮膚疾患との肉眼的鑑別も容易ではない。鑑別診断、治癒判定およびレトロスペクティブな疫学調査には血清学的診断が有効である。今回、エクアドルの皮膚リーシュマニア症患者の血清を材料として、ウエスタンブロット法による血清診断と抗原分析を行った。

エクアドルでは *L. mexicana* 群と *L. braziliensis* 群の原虫が地域別に分布していることをこれまでに明らかにしてきた。そこで、*L. amazonensis (La)*と *L. panamensis (Lp)*を抗原用原虫種として選択し、promastigote の可溶性抗原を 5-20% SDS-PAGE ミニゲルで展開し、各流行地の患者血清についてイムノブロットを行った。その結果（１）汎発性皮膚型の患者血清ではきわめて多数のバンドが検出された。（２）*L. panamensis* 流行地の患者血清は *La* より *Lp* 抗原と強く反応した。抗原バンドの数は数本程度と少なく、120, 100, 80 kDa あたりの分子と 15kDa 以下の低分子が主な抗原としては認識された。（３）*L. mexicana* 流行地の患者血清では明瞭なバンドが観察されなかった。このことから、小さい皮膚病変を形成し自然治癒する本地域の *L. mexicana* 感染に対しては、抗体産生が活発でないことが示唆された。（平成 14-17 年度、基盤A海外）