

## Letters to the Editor

### HTLV-1 Non-Saudi blood donors at King Fahd General Hospital, Jeddah

*To the Editor:* Human T-cell lymphotropic virus type 1 (HTLV-1), the first discovered human retrovirus, is a blood-borne pathogen that has been determined to be the cause of adult T-cell leukemia and lymphoma (ATL) and tropical spastic paraparesis (TSP, also referred to as HTLV-associated myelopathy). Limited studies also suggest its association with a number of other hematological malignancies, including polymyositis, polyarthritis, and uveitis.<sup>1-4</sup> HTLV-1 is now recognized to occur worldwide, although it is characteristically endemic in certain foci in Japan, the Caribbean, Southeastern USA, South America, Central Africa, Seychelles, and certain areas of Asia. The number of infected people worldwide has been estimated at 10-20 million,<sup>3</sup> with 1.2 million in Japan alone. HTLV-1, or its associated diseases, has now been detected in several countries in the Middle East, including Iran,<sup>6</sup> Egypt,<sup>7</sup> Iraq,<sup>8</sup> Kuwait<sup>9</sup> and Israel.<sup>10</sup>

HTLV-2, a close relative to HTLV-1, has a lower incidence worldwide and its association with diseases is less certain, although it has been detected at increasing frequencies among intravenous drug abusers and normal blood donors in Western countries. Because of cross reactivity between HTLV-1 and HTLV-2, these viruses are detected simultaneously by screening tests using the enzyme immunoassay (ELISA), but can be differentiated thereafter by the Western blot or the polymerase chain reaction (PCR) techniques.

A few countries, including Japan and the United States, have enforced screening of all donated blood for HTLV-1 antibodies. Screening has been enforced in Japan since 1986,<sup>4</sup> and in the United States since 1988.<sup>11</sup> Other countries (e.g., France) apply selective screening of donors with previous history of residence in endemic areas.<sup>12</sup> HTLV-1 seroprevalence among blood donors was reported at 0.018% to 0.043%<sup>11</sup> in the US, at 0.011% in France,<sup>12</sup> but at the high rate of 3% in Nagasaki, Japan.<sup>4</sup> Significant rates were detected in Caribbean and South American countries (e.g., 1.5% in Trinidad and 0.4% in Brazil) and in certain countries in Africa (0.7% to 3.6% in Nigeria, 1.2% in Senegal, 1.6% in Liberia, and 0.1% in

TABLE 1. HTLV-1 screening of blood donors at KFGH, Jeddah, September 1995 to September 1996.

Total screened	7628
Confirmed positives	2
Indeterminate	11

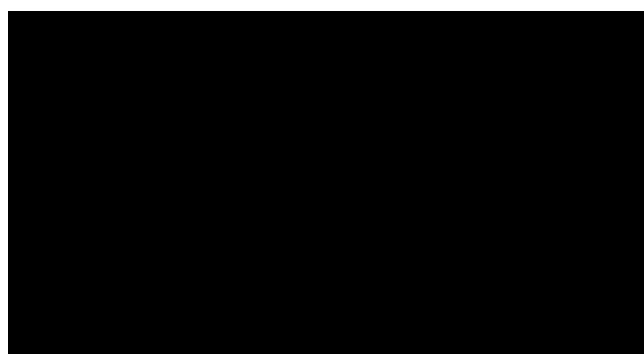
Zimbabwe).<sup>13</sup> Several studies from Europe (e.g., Sweden, Spain, Italy and Belgium), and some Asian countries

(Taiwan, Mongolia), reported no incidence among blood donors.<sup>1-5</sup> There are conflicting viewpoints on the need to screen donated blood for HTLV-1/2 in nonendemic areas, but most countries, to date, have not adopted a policy of routine screening for these viruses.

In Saudi Arabia, routine screening of blood donors for HTLV-1/2 was first adopted at King Faisal Specialist Hospital and Research Centre (KFSH&RC) in Riyadh in 1989, and two cases of indeterminate expatriate donors were reported.<sup>14</sup> A preliminary survey conducted by the Ministry of Health (MOH) in 1994 detected two cases of HTLV-1 positive expatriates among 9690 blood donors in various centers in Saudi Arabia (Dr. A. Al-Momen, personal communication). Currently, screening for HTLV-1/2 is carried out at several MOH and National Guard hospitals, as well as KFSH&RC. Screening of donated blood for anti-HTLV-1/2 antibodies at King Fahd General Hospital in Jeddah was started in July 1995 at the directive of the Ministry of Health. Over a one-year-period (July 1995-September 1996), 7628 donors were screened by the enzyme immunoassay test (Abbott, North Chicago, USA). Here we report the outcome of this work.

Two positive donors, one from India and the other from Ethiopia, were detected during the screening. These donors gave a high reading on repeated testing by ELISA. They were confirmed by Western blot (HTLV Blot 2.4 from Genelab Diagnostics, Singapore). The first donor was detected after the screening of 2700 samples, while the second one was detected after the number exceeded 7000 samples. Figure 1 shows the Western blot obtained with the blood of the first donor. The peptide pattern was indistinguishable from that given by the HTLV-1 positive control antiserum provided with the kit (including gp46 and recombinant rgp46). The same applies to the Western blot pattern of the second donor (not shown). These two donors are considered as confirmed positives for HTLV-1 antibodies. The two donors were completely asymptomatic and were negative for HBs Ag and for antibodies to hepatitis C virus, HIV and syphilis.

Eleven additional donors (including several Saudis) gave repeated intermediate readings on ELISA testing. They gave indeterminate results when Western blot was carried out and interpreted according to the manufacturer's instructions. Figure 1 shows a few indeterminate patterns obtained from some of the donors. Blood units giving indeterminate results, as well as those positive ones, were discarded. Table 1 summarizes the above-mentioned findings. To our knowledge, this is the first report of confirmed positive HTLV-1 cases among expatriate blood donors in Saudi Arabia. No cases have been detected as yet among Saudi donors.



**Jamal A. Maatooq, MD**  
**Malak Gazal, MBBS**  
**Manal Bawazeer, BS**  
 Laboratory Department  
 King Fahd General Hospital  
 Jeddah, Saudi Arabia

FIGURE 1. Western blot results of HTLV-1 ELISA-reactive samples. A: anti-HTLV-1 positive control serum; B: serum of first positive donor (#93095); C-F: sera of four indeterminate donors showing various reactive bands.

Our findings confirm the presence of positive cases among expatriate blood donors in Saudi Arabia. Such donors constitute a significant proportion of the local blood supply. In 1995, expatriates constituted 47.5% of the blood donors at KFGH, Jeddah (Egyptians 11.2%, Yemenis 8.8%, Sudanese 6%, Pakistanis 4%, Palestinians 3.3%, Indians 2%, Filipinos 1.7%, Syrians 1.7%, Indonesians 1.5%, Somali 1.4%, Bangladeshi 1.3%, others 4.6%).

Our rate of HTLV-1/2 confirmed positivity of approximately 0.026% is not much different from that of the USA. The relatively small number of HTLV-1/2 ELISA-positive donors (including indeterminates) indicates that wastage of blood units is unlikely to be a significant problem, although this will depend, to a great extent, on the type of kit used for screening. The indeterminate results require additional efforts in counseling and repeat testing of the donors. Donors giving consistently indeterminate results upon repeat screening must be considered negative for HTLV infection.

The cost effectiveness of HTLV screening of donated blood remains a difficult point and must be based on the prevalence of infection in the population under consideration as well as the available resources. The fact that there is a significant expatriate donor population in several centers of Saudi Arabia suggests that attention must be paid to infections not necessarily prevalent in the native population. Whether screening of donated blood for HTLV-1/2 may be limited to this category of donors requires further discussion. The possibility of encountering positive Saudi donors with relationship to known foci in the region must be considered. Continued surveillance and reporting of positive cases will help to provide a more precise evaluation of the situation in this country.

**Ghazi A. Jamjoom, PhD, FRCPath**

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## Tumor necrosis factor in falciparum malaria

*To the Editor:* Gandapur and Malik<sup>1</sup> have estimated the tumor necrosis factor (TNF) in falciparum malaria cases and correlated it with various clinical and laboratory parameters, and they have also provided literature support for their concordant and discordant association.

The other explanations for the vast variation are regulatory polymorphisms<sup>2,3</sup> of cytokine genes in the production of TNF, the central role of glycosylphosphatidylinositol (GPI) of parasitic origin for the severity of malaria,<sup>4</sup> strains of *Plasmodium falciparum*,<sup>5</sup> and sequestration of malaria pigment from parasites in the host microvasculature, which interacts with monocytes for

the induction of organ-specific or systemic pathophysiology of falciparum malaria.<sup>6</sup> The authors' future studies will throw more light on many of the hidden factors in malaria.

As cerebral malaria occurs in only small proportions of infected individuals, the benefits of TNF response to the malarial toxin probably outweighs its disadvantages<sup>7</sup> for the general population. Moreover, TNF progressively decreased seven days after treatment<sup>8</sup> even in those with severe disease. TNF is also produced to reach high levels during *Plasmodium vivax* infection.<sup>9</sup>

Finally, a comparison of the TNF alpha of healthy subjects with values found in other countries would have been interesting and informative, as it would have shown the levels in subclinically exposed population in a malaria endemic area from non-exposed population. The scientific community would be interested in this observation.

**P. Thirumalaikolundusubramanian, MD**  
King Fahd Central Hospital  
Gizan, Saudi Arabia

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### Reply

*To the Editor:* The interest of Dr. Thirumalaikolundusubramanian in our above article is highly appreciated. He has rightly mentioned that many factors are involved in the production of TNF in patients with severe falciparum malaria.

Although the incidence of cerebral malaria is small, it is a major cause of morbidity and mortality in tropical and sub-tropical countries. There is, therefore, the need to prevent and treat this dreadful complication. The following measures may be adapted in the future: 1) Development of

monoclonal antibodies against TNF and use of these antibodies as therapeutic agents in the treatment of acute severe falciparum malaria, particularly CM. The combined use of antibodies against TNF, IL-1 and IL-6 may be of immense clinical importance; 2) development and use of synthetic analogues of TNF which may have host-protective effect, if the potential deleterious effects of TNF are eliminated by using selected characterized subunit of the cytokine; 3) development of drugs that decrease TNF release from macrophages via the inhibition of phosphodiesterase and the increase of cyclic AMP, which may act as supportive agents in the treatment of acute falciparum malaria.

*Plasmodium vivax* does not produce CM. TNF is responsible for the rosetting and cytoadherence phenomena in CM. Such pathology does not occur in vivax malaria.

The TNF level in our normal healthy control subjects was 108.08 pg/mL, while the TNF level in our malaria patients in adults and children was 117.8 pg/mL and 149.0 pg/mL. This means that the TNF level in healthy subjects and mild malaria patients did not differ significantly. The TNF levels reported by previous workers are shown in Table 1.

The comparatively higher levels of TNF in our situation are probably due to the presence of immunity in the community studied, one explanation being that none of our hyperparasitemic patients (range: 100,000-558,000 parasites/ $\mu$ L of blood) died. Again, the incidence of severe complications was much less in our setting than previously reported.

TNF is one of the defence tools of the body. Only if produced in excess will it have harmful effects. So there is the need to study the immunological profile of our patients and the genetic make-up of concerned malarial parasites. These findings may be helpful in the prevention and

TABLE 1. TNF levels reported by various workers.

Authors	Year	TNF pg/mL	Countries
Gandapur and Malik <sup>1</sup>	1996	108.08	Pakistan
Ringwald et al. <sup>2</sup>	1993	6.0	Madagascar
Butcher et al.	1990	undetectable	Solomon Island
Saisy et al. <sup>3</sup>	1994	38.6	West Africa
Kwiatkowski <sup>4</sup>	1995	80.0	Gambia

treatment of falciparum malaria.

**Abdus Salam Khan Gandapur, MBBS, MPhil**  
Department of Basic Medical Sciences  
Gomal University  
Dera Ismail Khan, Pakistan  
**Salman A. Malik, MSc, PhD**  
Department of Biological Sciences  
Quaid-I-Azam University  
Islamabad, Pakistan

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### Frequencies of a C-T Mutation in Lipoprotein Lipase and a G-A Mutation in Apolipoprotein-A1 in Saudi Arabia

*To the Editor:* Lipoprotein lipase (LPL) is a key enzyme involved in the hydrolysis of triglyceride rich lipoproteins. Pro to Leu substitution as a result of C-T transition in the codon 207 is the basis for lipoprotein lipase deficiency in the Quebec region of Canada, where its frequency is 1 per 40.<sup>1</sup> This deficiency is associated with hyperlipidemia, which accounts for some of the causes of coronary heart disease.<sup>2</sup> The screening for this mutation was undertaken within the framework of our program to identify markers of significance to the disease for detecting individuals at risk. Likewise, a G to A mutation, 76 base pairs upstream from the transcription start site of apolipoprotein AI, was included in this program. This mutation has been shown to affect the capability to adjust to changes in monounsaturated fatty acids in dietary fat.<sup>3</sup> Previous studies have associated changes at this locus with dyslipidemias and coronary heart disease.<sup>4,5</sup> While this mutation occurs at a frequency of 0.2 in Caucasians, figures are not available for the Saudi population. This report summarizes our results in the screening of Saudi subjects.

Seven mL blood samples were collected in tubes with EDTA. Peripheral blood leukocytes were isolated by the Ficoll-Paque method. From these cells, DNA was extracted by a salting-out procedure.<sup>6</sup> For LPL Pro-Leu mutation, a fragment of 210 bp was amplified from the genomic DNA,

FIGURE 1. Amplification of a fragment of lipoprotein lipase containing the codon 207 for proline. The primers used in the polymerase chain reaction were capable of creating a recognition site for the restriction enzyme DdeI, if a C-T transition had taken place. The fragments remained undigested. The control for digestion has not been shown. The M lane shows the HaeIII digested DNA from the replicative form of OX174.

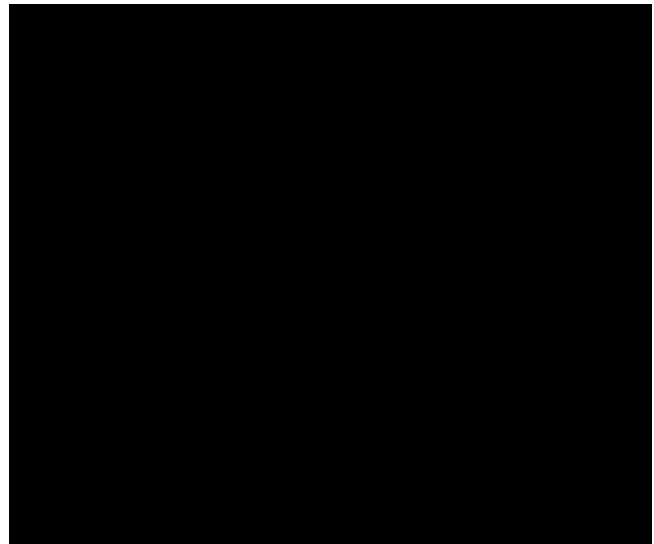


FIGURE 2. Amplification of a 432bp fragment from the 5' region of apolipoprotein AI. The fragment remained undigested with the restriction enzyme MspI, as none of the subjects had a G to A mutation.

using primers and the PCR amplification conditions suggested by Bijvoet and Hayden.<sup>1</sup> The amplified fragment (Figure 1) was digested by the restriction enzyme DdeI. Phage DNA was used in both assays as a positive control for the restriction digests. This mutation was not observed in the 100 Saudi subjects. Accordingly, we estimated that the frequency of this base change was below 1 per 100.

For the G to A mutation, creating an MspI recognition site on the 5' side of apolipoprotein AI, a fragment of 432 bp was amplified by PCR (Figure 2) using the same primers as were used by Lopez-Miranda et al.<sup>3</sup> A total of 70 subjects were screened and the RFLP site was not found in any of the subjects. This observation suggests that the frequency of the rare allele in the apolipoprotein-AI gene is less than 1 in 70, which is more than 14 times lower than that in Caucasians.

It may be concluded from these results that the Saudi population is different from the Caucasian population as far as these two polymorphic sites are concerned. Any future studies aimed at utilizing these sites for marker purposes would not be expected to yield any useful association data in this population unless very large numbers are screened.

**Jehad Buraiki, MD**  
**Abdul Islam Butt, M.Phil**  
**Syed Saeed Hussain, PhD**

Department of Cardiovascular Diseases  
 Department of Biological and Medical Research  
 King Faisal Specialist Hospital & Research Centre  
 Riyadh, Saudi Arabia

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### The Role of Ultrasonography and Ultrasound-guided Aspiration in Blunt Abdominal Trauma

*To the Editor:* I read with great interest the article on the above subject published in the *Annals* recently, by El-Abdullah et al.,<sup>1</sup> and felt obliged to make the following comments.

First, it was not clear whether the article was a prospective or a retrospective study. Although the authors mention that there were 3000 consecutive patients examined in a period of five years, giving the impression that this was a prospective study, the protocol of the study is similar to that of Goletti et al.,<sup>2</sup> including the algorithm. Actually they had similar results by performing ultrasound within 30 minutes of patients' arrival, with an average of four minutes to perform it. Was this a retrospective or prospective analysis?

Second, when evaluating any test, a gold standard is needed to compare the results. Although laparotomy was used as the gold standard for the operated patients, the authors did not define the false-positive findings for the non-operated patients, who also need a gold standard (CT scan, for example). This is important when studying the specificity. Specificity is defined as the true-negative results divided by the summation of true-negative and false-positive. The authors mentioned that ultrasound has a specificity of 97% in detecting splenic injury. Since there were 2927 true-negative results, a simple calculation shows that there were 91 false-positive splenic injuries. How were these defined, and what was their clinical course?

Third, the authors cited only four references relating to the use of ultrasound in blunt abdominal trauma, which is a controversial topic. One of these was wrongly cited,<sup>2</sup> as the authors mentioned that sensitivity, specificity and accuracy in ultrasound in detecting hemoperitoneum approach 86.6%, 100% and 97% respectively. The original reference shows that "the overall sensitivity of US in detecting free fluid collection in that study was 98%, with a specificity of 99% and a positive predictive value of 100%." Extensive literature has been published over the last four years regarding the role of ultrasound in blunt abdominal trauma which could have been evaluated. I notice that the time between finishing the study (April 1995), and its submission (May 1995), was less than two months. This is a very short period compared to this extensive experience (3000 patients). It is actually the largest series in the literature reported to date. The second largest series was reported by Glaser et al.,<sup>3</sup> and was a retrospective analysis of 10 years' duration, involving 1151 patients. Writing a clinical study should take a lot of consideration, including critical evaluation of the information available.

The fourth point is that the inclusion criteria of the study were not mentioned. This is essential in defining the study population. The reported injury percentage of this study (true-positive and false-negative findings) is the lowest in the literature at 2% (73/3000), compared to other studies which varied between 6% to 54%.<sup>3-7</sup> Was ultrasound actually indicated in all these patients?

My last point is that it seemed that some of the statistical principles are not clear to the authors. The mean (average) is a single number which is a summation of all the observations in a set of data divided by the total number of measurements. It cannot be three to four minutes, as mentioned in the discussion.

I am aware that criticizing a study is easier than performing it, but it is always advisable to have a discussion with experts in research methodology before implementing any study. There has been a great deal of concern about the knowledge of surgeons in research methodology.<sup>8</sup> Some have even gone further to ask readers to be cautious when interpreting the results of surgical trials.<sup>9</sup> Readers should take the responsibility of critical evaluation of published articles. This is an important part of information literacy, which is defined as the ability to locate, critically evaluate, and use the information available.

**Fikri Abu-Zidan, MD, FRCS, PhD**  
 Senior Research Fellow  
 Department of Surgery  
 Auckland Hospital  
 Faculty of Medicine and Health Sciences  
 University of Auckland  
 Park Road  
 Auckland, New Zealand

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### Reply

*To the Editor:* We are grateful to Dr. Abu-Zidan for his comments and queries. We welcome his comments and are ready to answer his questions as a scientific discussion.

With regards to his first comment, I would like to mention that our study was both prospective and retrospective. It was prospective in the form of implementing the policy of performing US and US-guided aspiration from 1990 as a screening test in multiple trauma and blunt abdominal trauma. In fact, we performed US of the abdomen as a routine in trauma cases long before 1985, but have made it obligatory since 1990.

The policy of conservative treatment for the stable cases depending on US and US-guided aspiration only started from 1990 in our hospital, but before that the policy was to open any hemoperitoneum. This explains our skill in performing US of the abdomen in the short time of three to five minutes, and immediately within 30 minutes of patients' arrival. This was going on before Goletti published his paper in 1994.<sup>1</sup> The retrospective aspect of the paper is the classification of the data, for which we admit to borrowing the algorithm from Goletti to represent our results.

With regards to the second comment, it is not practical to do a CT scan for negative cases (2927) just to confirm the US findings. We depended on experience gained from performing US in a lot of the trauma cases we saw. Despite the availability of CT in our hospital, we still use US and US-guided aspiration in screening the patient under investigation, and in the follow-up of patients on conservative treatment.

Our calculation and reference for true-positive and true-negative depended on the laparotomy findings. The positive predictive test was 96%, which means that the false-positive was one case only, and the true-negative cases were 32.

We agree with Dr. Abu-Zidan that there was a mistake in the reference quoted in the last paragraph of the introduction. The said reference should have been No. 2, Huang et al., instead of No. 6. The number of references may be limited, but we believe the article is still informative.

With regards to the number of cases screened being small as compared to total trauma cases, we are seeing between 1400 and 1500 RTA cases, and performing an average of 500 abdominal ultrasounds per year. US is complementary to clinical examination, and to avoid missing an abdominal injury we use it extensively. Besides this, however, there are many patients who present with blunt abdominal trauma, and who are observed and discharged home, depending on the clinical evaluation.

Regarding Dr. Abu-Zidan's fifth point, we did not measure the time of the US and take its mean, but we refer to the average time only.

Finally, we are grateful to Dr. Abu-Zidan for his comments, and to the editors for giving us a chance to clarify the issues raised.

**Dr. Hassan D. I. El-Abdullah**

Consultant Surgeon

**Dr. Anil Kumar Shukla**

Head of Radiology

King Khalid General Hospital

Hafr Al Batin, Saudi Arabia

### The spectrum of diffuse and focal liver lesions in workers exposed to vinylchloridemonomer

*To the Editor:* The awareness of the harmful effects of vinylchloridemonomer (VCR) on the liver dates back to 1948, but the real interest of the world was aroused in 1974, after Creech and Johnson's description of the onset of the liver angiosarcomas (ASL) in workmen employed in the vinyl and polivinylchloride industry (VC/PVC).<sup>1</sup>

In a retrospective analysis, the medical records of workmen from a VC/PVC plant, treated at the Clinical Hospital in Split, Croatia, from 1979 to 1988 (when the last emitter suspension-polymerisation plant in this factory was closed), were assessed. These included over 30,000 case histories, and the histories of 128 workmen from the "Jugovinil" plant. In workmen who consented to liver biopsy, histologic features were re-evaluated, while autopsy and histological findings were analyzed for workmen who died during the study period. The average concentrations of VCM in the working environments of the patients in question were measured.

Out of 31,395 patients admitted to hospital in that period, 128 (0.4%) were workmen from the plant (111 males and 17 females). A number of these workers (32, or 25%) were treated because of liver and/or gastrointestinal disease, as shown in Table 1.

Tamburro et al.<sup>2</sup> described the earliest changes in workmen in the VC/PVC industry exposed to VCM in the form of focal hepatocellular and mixed hyperplasia (hepatocytes and sinusoidal cells) as precursors of further liver lesion. However, on the basis of histologic changes alone, liver lesions cannot be attributed exclusively to VCM, as numerous other noxae (degeneration, steatosis, infectious hepatitis, granulomas, alcoholic and other toxic lesions of the liver) can yield a similar histologic picture. The near impossibility of a definite answer imposes the policy of regular and systematic check-ups of each workman with the registration of VCM exposure in adequate file cards, the use of proper dosimeters ("personal monitoring"), as well as regular measuring of the VCM concentration of the working environment ("area monitoring").

Another considerably more severe liver lesion caused by the exposure to increased concentrations of VCM is fibrosis, which may lead to liver cirrhosis. Connective tissue hyperplasia in septal and portal spaces was a dominant characteristic in our patients, sometimes with elements of cirrhosis with anisocytosis, binucleation, fat droplets and isolated hepatocyte necrosis. The differentiation from alcoholic lesions may be difficult, and

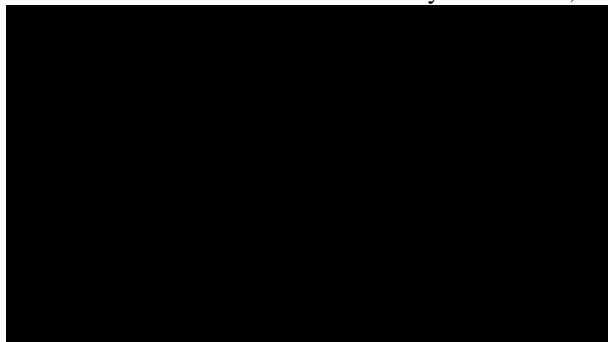


FIGURE 1A. VCM toxic hepatitis: liver parenchyma of preserved architecture, hepatocytes uniform, which round regular nuclei, in some places anisonucleosis. There are hepatocytes with two nuclei as the sign of regeneration. Cytoplasm moderately abundant, with grains of bile pigment. Few scattered hepatocyte necroses. Portal spaces of adequate width, scarcely infiltrated with mononuclear inflammatory cells. B. VCM liver fibrosis: markedly hyperplastic connective tissue, partly infiltrated with mononuclears, a few preserved hepatocytes which are regular, with moderately large cytoplasm, while the nuclei show moderate anisocytosis.

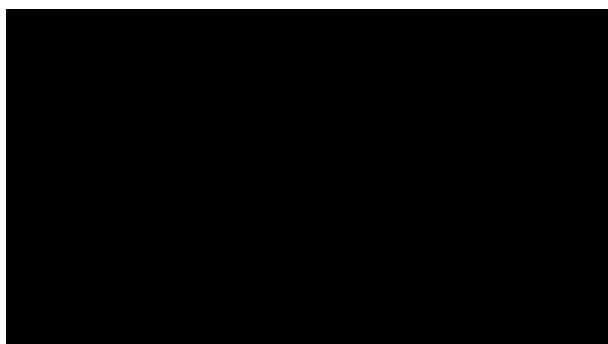


FIGURE 1C. VCM liver cirrhosis: liver parenchyma of disordered architecture, portal spaces with hyperplastic connective tissue are naturally communicating, forming pseudolobules. Hepatocytes show moderate anisocytosis, and occasional binucleation. Fat vacuoles, isolated necroses of hepatocytes can be seen in places. D. VCM liver angiosarcoma (ASL): dense rows of tumor cells can be seen in the areas of hemorrhage, the tumor vascular space is lined with atypical epithelium; markedly sarcomatous stroma, dense rows of tumorous cells with distinct polymorphism and hyperchromatosis.

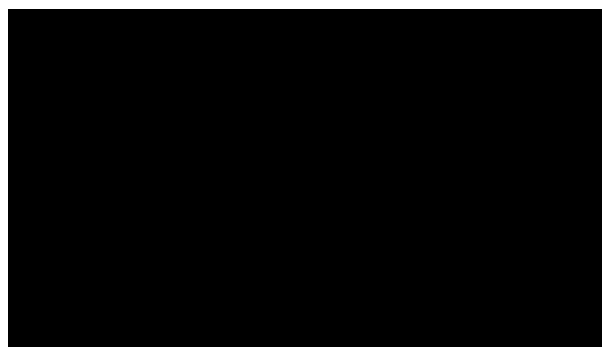


FIGURE 1E, F. VCM hemangiopericytoma (HEPC) of the liver: tumor tissue consisting of atypical pericytes nests is mostly in rows on the outer side of the vessel; the cells are often anaplastic with numerous pathologic mitoses and large areas of necrosis (E). Reticulin fibers can be seen by means of silver staining (F).

TABLE 1. *The spectrum of liver lesions caused by VCM.*

Diagnosis potentially related to VCM	Number
Hepatitis chronica (toxica)	15
Cirrhosis (fibrosis) hepatis	14
Angiosarcoma hepatis (ASL)	2
Hemangiopericytoma hepatis (HEPC)	1
Total	32

the patient's history is often of crucial importance.

Popper<sup>3</sup> and Gedigk et al.<sup>4</sup> have undoubtedly given the greatest contribution to histogenesis and pathohistology of liver angiosarcoma (ASL). The assumption is that the onset of ASL includes proliferation of sinusoidal cells and sinusoidal dilatation, leading to atypical hyperplasia, while direct stimulation of hepatocytes by VCM leads to proliferation of hepatocytes, formation of regenerative nodules and hepatomas. The tumor may develop in a papillary form, where large vascular spaces and systems of anastomosed blood lined with atypical endothelium are embedded in a sarcomatous stroma.<sup>5</sup> Once started, the development of the tumor is irrepressible, regardless of further exposure to VCM.<sup>6</sup>

Hemangiopericytoma (HEPC) is a rare malignant tumor formed of pericytes, cells that wind around the capillaries. It metastasizes in approximately 12% of the cases, and otherwise develops rather slowly, pushing aside the

adjacent structures with no excessive discomfort. The most frequent way of spreading is lymphatic. The typical picture of HEPC is characterized by rich vasculature consisting of endothelial canals surrounded by spindle-shaped, oval, or round cells with marked nuclei. In HEPC the cells are arranged around the basal membrane with collapsed capillaries and preserved reticulin stroma, while in ASL the endothelial cells proliferate and interrupt the reticulin stroma. Since the pericytes are separated from endothelial cells by the basal membrane only, the reciprocal transformation of pericytes into endothelial cells and vice versa, which would favor the casual interrelation between HEPC and ASL, has been speculated.<sup>7</sup> Increased cellularity and intensive mitotic activity, in addition to areas of necrosis and hemorrhage, are characteristic of HEPC malignancy,<sup>8</sup> especially if necrotic areas are conspicuous. It is to be assumed that in the polymerization of VCM, not only ASL but also other types of tumor of very similar vascular etiology may occur, usually in the secondary processing of the PVC powder which still contains some residual VCM, even after the process of stripping (i.e., removal of remainder VCM<sup>9</sup>) has been carried out.

**Izet Hozo, MD, PhD**  
**Zvonko Rumboldt, MD, PhD**  
**Šimun Anđelinović, MD, PhD**  
**Dragan Ljutić, MD, PhD**  
**Lovre Bojic, MD, PhD**  
**Dinko Miric, MD, PhD**  
 Clinical Hospital  
 Spinciceva 1  
 21 000 Split, Croatia

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### Rapid Diagnosis of *Mycobacterium tuberculosis* by Amplicor PCR and LCR in a Clinical Laboratory

*To the Editor:* Saudi Arabia still has an incidence of tuberculosis two to four times higher than most developed countries, despite the great decline from 135 per 100,000 in 1980 to 18.6 per 100,000 in 1990, the highest figures having been reported from the Jeddah health region.<sup>1</sup> Due to the communicability of tuberculosis, the delay in obtaining confirmed diagnosis by culture contributes to the spread of the disease, therefore, as an alternative to culture, methods amplifying mycobacterial nucleic acid have been developed which can reduce the time for diagnosis to a single day.

In our patient population, patients with positive acid-fast bacilli (AFB) smears and pulmonary symptoms, with or without radiological findings, are frequently started on antituberculosis therapy, pending the results of culture, which may take up to 12 weeks. It has been observed in the Southern region of Saudi Arabia that up to 50% of healthy individuals harbor non-tuberculosis mycobacteria (NTM) in their mouths and throats.<sup>2</sup> The high incidence of NTM complicates the diagnosis of tuberculosis, and frequently results in the unnecessary administration of antituberculosis drugs. Therefore, tests which provide a high specificity for MTB in smear-positive specimens will prove cost effective in reducing the use of antituberculosis drugs, contact investigations and isolation facilities.

Isothermal enzymatic amplification targeting repetitive sequences in ribosomal ribonucleic acid (rRNA), using the Gen-Probe Amplified *Mycobacterium tuberculosis* Direct Test (MDT) and Amplicor polymerase chain reaction (PCR), which uses PCR to amplify a deoxyribonucleic acid (DNA) target sequence in the 16S rRNA gene, have now been widely applied for the detection of *M. tuberculosis* (MTB) nucleic acid in the processed sediments of respiratory specimens. Although these techniques are more sensitive than direct microscopy, discrepant results occur between these molecular methods and conventional culture.<sup>3</sup> Ligase chain reaction (LCR), a probe amplification technique first described in 1989 and modified in 1991 by incorporating a DNA polymerase, is based on sequential rounds of template-dependent ligation of two adjacent oligonucleotide probes.<sup>4</sup>

A comparative study between the sensitivity and specificity of the LCR and Amplicor PCR assays for MTB with culture on respiratory specimens, collected between April and August 1996, was conducted in our Microbiology Division. Three hundred and twenty-six patient samples were selected for this study on the basis of positive smear for AFB, documented history of tuberculosis on treatment, clinical suspicion of tuberculosis with negative microscopy, or on the basis of positive culture. Each sample, in addition to microscopy and culture, was tested by both Amplicor PCR and LCR. Specimens were decontaminated by the standard *N*-acetyl-L-cysteine sodium hydroxide method and

concentrated by centrifugation at 3000 x g for 20 minutes. The final pellet was resuspended to 1.5-2.0 mL in sterile saline. Each specimen was inoculated onto Lowenstein-Jensen slopes and MB/BacT Middlebrook 7H9 broth, and cultures were monitored for eight weeks. An aliquot from each sample was stored frozen at -20°C for up to four months until molecular assays were performed. Of the processed sediment, 0.1 mL was assayed by the Amplicor PCR *Mycobacterium tuberculosis* test (Roche Diagnostic Systems, Branchburg, N.J.) according to the manufacturer's instructions. 0.5 mL of the sediments from processed clinical specimens were tested by the LCR *Mycobacterium tuberculosis* assay (Abbott LCx Probe system, Abbott Diagnostics, Chicago, Ill.) according to the manufacturer's directions.

The results of the 326 samples are given in Table 1. Of the 74 specimens which were culture positive for MTB, 46 (62%) were AFB smear positive. All the 46 smear-positive, MTB-positive cultures were LCR positive (100%) and 44 (95.6%) were positive by Amplicor PCR. Of the 28 smear-negative, MTB-positive cultures, 25 (89.2%) were LCR

TABLE 1. Culture, LCR, amplicor PCR and smear results.

Mycobacteria culture and AFB smear results	# Samples	# LCR pos.	# Amplicor PCR pos.
MTB (AFB smear pos.)	46	46	44
MTB (AFB smear neg.)	28	25	21
All MTB pos. cultures	74	71	65
All NTM	68	0	0
No growth	184	4	2
Total	326	75	67

positive and 21 (75%) were Amplicor PCR positive. Of all specimens, 68 (20.9%) were culture positive for NTM, and all were negative by Amplicor PCR and LCR. The sensitivity and specificity of LCR in the smear-positive samples were 100% and 97.4% respectively. In smear-positive specimens, Amplicor PCR showed a sensitivity of 95.6% and specificity of 98.7%. The high sensitivity and specificity of both the Amplicor PCR and LCR assays in the smear-positive specimens suggest that both can detect and differentiate MTB from NTM in a single respiratory specimen, thereby greatly reducing the time of diagnosis of pulmonary tuberculosis. In contrast, the sensitivity of both assays was diminished in smear-negative specimens. The sensitivity of the LCR and Amplicor PCR assays was reduced to 89.2% and 75% respectively in smear-negative specimens, however, the specificity of both assays remained high at 98.8% and 99.4% respectively. These highly favorable preliminary results in this study for LCR suggest that a negative result will almost certainly exclude the possibility of MTB in adequately collected respiratory samples. Both Amplicor PCR and LCR will differentiate equally and accurately between MTB and NTM, which

increases the reliability of these techniques. In patients with negative smear and radiological evidence of disease, it would be advantageous to test multiple (at least two or three) respiratory samples, to reduce the risk of a false-negative result due to the low numbers of organisms, particularly in severe immunocompromised AIDS population where AFB smear has low sensitivity.<sup>5</sup> The LCR and Amplicor PCR-positive but culture-negative results (Table 1) observed in specimens from two patients who previously had positive cultures and had antituberculosis therapy, support the findings of other investigators that DNA amplification techniques can detect both viable and nonviable organisms.<sup>6</sup> This suggests that the clinical utility of these methods in monitoring patients on antituberculosis therapy is limited.

In both methods, sample preparation is not technically demanding, although specimens must be initially processed in a Class 1 Biological Safety Cabinet with strict adherence to the prescribed methodology. In our study, and as previously reported, Amplicor PCR is easily performed, and results can be reported within one eight-hour shift. Similarly, in the LCR assay, a batch of 48 specimens, including controls, can be prepared within 2½ hours. The amplification and detection steps are automated, and require approximately one hour for each step. It is therefore possible to incorporate this assay into the normal laboratory work flow and report results within one eight-hour shift.

In this study, LCR assay for MTB shows greater sensitivity than the Amplicor PCR assay in smear-negative specimens. The high specificity of both assays is of great benefit in differentiating MTB from NTM. In practical terms, the use of DNA amplification techniques allows for rapid and specific diagnosis of MTB eliminates unnecessary administration of antituberculosis therapy and the need for isolation measures for NTM patients, as well as delayed availability of species identification by cultural methods.

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**Christopher Gilpin, B. App.Sc.**  
**Mohamed A. Abdelaal, FRCPATH**  
**Abimbola O. Osoba, FRCPATH**  
 Department of Pathology  
 King Khalid National Guard Hospital  
 P.O. Box 9515  
 Jeddah 21423, Saudi Arabia

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