Serology and serum DNA detection in shingles

Marinko Dobec[a], Walter Bossart[b], Franz Kaeppeli[a], Juerg Mueller-Schoop[c]

[a] medica, Medizinische Laboratorien Dr. F. Kaeppeli, Zurich, Switzerland
[b] Institute of Medical Virology, University of Zurich, Zurich, Switzerland
[c] Permanence Hauptbahnhof Practice, Zurich, Switzerland

Summary

Aim: To investigate the sensitivity of various laboratory approaches in the diagnosis of herpes zoster from patient serum.

Methods: Paired sera from 53 consecutive adult patients with acute herpes zoster were tested for the presence of varicella-zoster virus (VZV) antibodies. All acute sera were tested subsequently by real-time polymerase chain reaction (PCR) for the presence of VZV DNA. In addition, convalescent sera of patients who tested initially positive for VZV DNA underwent PCR analysis.

Results: VZV IgM antibodies were found by enzyme immunoassay (EIA) in 5 acute (9%) and 20 convalescent (38%) zoster sera. VZV DNA was detected by PCR in 21 (40%) acute zoster sera and was no longer detectable in the convalescent samples. A seroconversion or a fourfold or greater titre increase was found by complement fixation (CF) test in 41 (77%), by IgG indirect fluorescent antibody assay (IgG IFA) in 43 (81%) and by CF and IgG IFA combined in 45 of 53 (85%) paired zoster sera. The combination of all serological methods detected 51 (96%) and PCR combined with serology identified 52 (98%) of 53 patients.

Conclusions: Optimal laboratory sensitivity in the diagnosis of herpes zoster from serum can be achieved by the combination of PCR and serology of paired serum samples. Serological methods alone are of limited value for early diagnosis of zoster when therapy can be initiated, because CF and IgG IFA need convalescent serum and IgM test sensitivity is insufficient. Early diagnosis of VZV reactivation is possible from serum by PCR in the first days of illness and test sensitivity needs further improvement. The findings highlight the need for future studies into the usefulness of PCR and serology in atypical cases of VZV reactivation.

Key words: VZV serology; DNA detection; PCR; shingles; VZV reactivation; herpes zoster

Introduction

Varicella-zoster virus (VZV) is the causative agent of *varicella* (chickenpox) during the primary infection in susceptible individuals. After primary infection, the virus establishes a lifelong latent infection in dorsal root ganglion cells. Its reactivation gives rise to *herpes zoster* (shingles), which is usually characterised by unilateral neuralgia followed by vesicular eruptions in a dermatomal distribution [1].

However, in certain circumstances and in special populations, both primary infection and VZV reactivation may present unusual manifestations such as asymptomatic *varicella* or subclinical reactivation [2–4]. Some patients experience *zoster* with the "*zoster*-type" pain in a dermatomal distribution but without the zosteriform rash known as *zoster sine herpete*. Many other unusual manifestations of VZV reactivation, such as Ramsay Hunt syndrome, facial nerve palsy without skin lesions, cerebellar ataxia, disease in the central nervous system and visceral *herpes zoster*, have been described [5–8]. In such conditions, clinical diagnosis may be very difficult and there is a need for strong laboratory support.

Although most clinicians readily recognise the signs and symptoms of *herpes zoster*, additional laboratory confirmation is frequently requested.

Confirmation of the diagnosis can be obtained by immunofluorescence or immunoperoxidase staining of vesicle scraping or viral culture of vesicle fluid [9, 10]. However, the sensitivity of these diagnostic methods is limited and highly de-

This study was supported by a grant of the Gianni Rubatto Foundation.

pendent on the quality of scrapings, handling time of vesicle fluids, and stage of the skin lesions.

Furthermore, in cases of *zoster sine herpete* and *zoster visceralis*, virus isolation can be extremely difficult to perform because vesicles are not available [8, 10].

Nevertheless, serodiagnosis of VZV reactivation remains useful in confirming the clinical diagnosis, particularly when vesicle specimens are of poor quality or not available at all, which is usually the case in routine diagnostic laboratories.

The majority of laboratories therefore perform serological IgM antibody determination and look for a rise in IgG or CF antibody.

The diagnostic importance of immunoglobulin M (IgM) response to VZV in reactivated infection, and serological cross reactions with other herpes viruses, are still controversial [11–13]. However, since a diagnostically relevant antibody response takes several days and depends on a functioning immune system, it may be less useful when a rapid decision regarding antiviral therapy is necessary.

Although PCR has recently been used for the diagnosis of VZV reactivation from cerebrospinal fluid, whole blood, plasma, serum and saliva, it was rarely compared to serology [9, 10, 14].

The aim of the present study was therefore to investigate the performance of VZV serology in comparison to VZV load in the serum of patients with *herpes zoster* in the course of clinical illness, and to evaluate the benefits of this diagnostic approach for laboratory diagnosis of herpes zoster.

Of further interest would be the evaluation of methods for VZV antibody and DNA detection from patient serum in atypical cases without skin eruptions and with an unusual clinical presentation.

Material and methods

Patients

The study included paired sera from 53 consecutive otherwise healthy adult patients (26 females and 27 males, age-range of subjects 26–84 years) with acute *herpes zoster*. Clinical diagnosis was based on the typical morphology and distribution of the skin lesions and included patients with *herpes ophthalmicus* (7 patients), *oticus* (one patient), *cervicalis* (9 patients), *thoracalis* (25 patients), *lumbalis* (10 patients) and *sacralis* (one patient); no atypical zoster cases were included. All sera originated from patients who attended a primary health care unit at Zurich main railway station (Permanence Clinic Hauptbahnhof Zurich) for medical diagnosis and treatment. Serum samples were collected, after informed (verbal) consent at their first visit (acute sera; 0–21 days after the onset of initial clinical symptoms) and at a follow-up visit (convalescent sera; 7–40 days after collection of the acute samples). The sera were kept frozen at –20 °C until the serological tests were performed.

Methods

– *DNA preparation:* DNA for PCR analysis was extracted from the patient sera by the MagNa Pure Nucleic Acid Extraction System (Roche Molecular Diagnostics). Alternatively, DNA was extracted and purified on silica spin columns (QIAamp DNA mini kit, Qiagen), according to the manufacturer's instructions. Both methods had previously proven to be equivalent with respect to the extraction of DNA from bloodborne viruses.
– *Amplification and detection of DNA:* Real-time PCR was performed using standardised TaqMan® chemistry and ABI 7300 real-time PCR systems (ABI Applied Biosystems, Forster City, CA, USA).The VZV glycoprotein I (gpI) gene (ORF 67 of the unique small segment of the VZV genome) served as target sequence. The oligonucleotides used for amplification and detection were: 5'-ACAGCTTGTCTT-TATTGGAGAGCAA-3' as forward primer, 5'-GC-CACCGTATCCGCGTATA-3' as reverse primer and 5'-(FAM)ACCTACCGGGACAAACTATAGC-GGAACACTG(TAMRA)-3' as the TaqMan® probe. Real-time PCR was performed on all acute *zoster* and on convalescent sera of those patients who tested initially positive for VZV DNA by PCR, as described elsewhere [15].
– *Serology:* Paired sera from 53 adult patients with acute *herpes zoster* were analysed for the presence of VZV antibodies by IgG indirect fluorescent antibody test (IFA Merifluor, Gull Laboratories, USA), IgM enzyme immunoassay (EIA Genzyme Virotech, Germany) and complement fixation test (CF; Institute Virion, Switzerland) according to the manufacturer's instructions. All serum pairs were examined in parallel at the same time.

Herpes simplex virus (HSV) IgM EIA (Genzyme Virotech, Germany) as well as Epstein-Barr virus (EBV) capsid antigen IgM IFA (VCA IgM IFA, Institute Virion, Switzerland) were performed to rule out cross reactions between between VZV, HSV and EBV in all patients who tested positive for VZV IgM.

To avoid false positive and false negative IgM results, IgG antibodies were removed by adsorption with the RF-Sorbo Tech reagent (Genzyme Virotech) prior to IgM testing according to the manufacturer's instructions.

Statistical analysis

For evaluation of the test results, a 95% confidence interval (CI) for proportions based on Agresti and Coull was applied [16].

Results

PCR analysis

VZV DNA was found in 21 (47%; 95% CI, 32.9–60.9%) of 45 samples collected within the first 8 days of illness and in 21 of the total 53 acute zoster sera (40%; 95% CI, 27.6–53.1%). It was no longer detectable in the convalescent sera of patients who tested initially positive for VZV DNA in the acute sera (Table 1).

Serology

VZV IgM antibodies were detected by EIA in 5 out of 53 (9%; 95% CI, 3.7 to 20.7%) of the acute sera (IgM1) and in 20 out of 53 (38%; 95% CI, 27.6 to 53.1%) convalescent sera (IgM2).

No IgM antibodies against HSV and EBV were detected in acute and convalescent sera of zoster patients who tested positive for VZV IgM antibodies (Table 2).

Seroconversion or a fourfold or greater increase of VZV antibody titre was detected by CF in 41 (77%; 95% CI, 64.3–86.7%) and by IgG IFA in 43 (81%; 95% CI, 68.4–89.6%) paired sera of *zoster* patients (Figure 1).

Combined PCR analysis and serology

Best performance was achieved using a combination of PCR and all serological methods [52 patients (98%; 95% CI, 89.1–100%) detected].

The combination of all serological methods and combination of IgM1, IgM2 and IFA detected 51 patients of 53 (96%; 95% CI, 86.5–99.7%), followed by the combination of PCR, CF and IFA (47/53; 89%; 95% CI, 77.1–95.1%). IgM1 and IgM2 in combination with CF detected 47 patients (89%; 95% CI, 77.1–95.1%) and PCR combined with IFA 46 patients (87%; 95% CI, 74.9–93.8%). Both combinations, CF and IFA as well as PCR and CF, detected 45 patients (85%; 95% CI, 72.7–92.4). PCR in conjunction with IgM1 and IgM2 identified 34 patients (64%, 95 CI, 50.7–75.7%), PCR and IgM2 34 patients (60%, 95% CI, 46.9–72.4%), PCR and IgM1 25 patients (47%; 95CI, 34.4.–60.3%) and IgM1 combined with IgM2 23 patients (43%; 95% CI, 30.9–56.7%).

The findings of the individual tests and their combinations are shown as proportions of positive testing with their 95% confidence intervals (Figure 2).

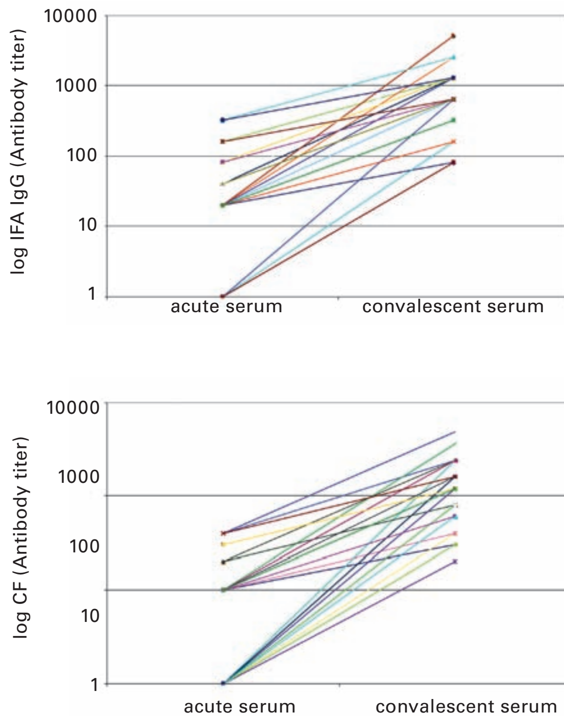

Figure 1

Immune response to *varicella-zoster virus* in acute and convalescent sera from 53 adult patients with acute herpes zoster, based on indirect fluorescent antibody IgG test (IFA IgG; top) and complement fixation test (CF; bottom). Acute and convalescent paired sera, in whom seroconversion or a fourfold or greater titre increase was observed [41 (77%) by CF and 43 (81%) of 53 samples by IFA IgG], are linked for each individual patient. Data for the analysis of antibody titres were log-transformed.

Table 1

Performance of PCR test for the detection of varicella-zoster virus DNA from patient serum in the course of herpes zoster.

PCR test result	Acute sera				Convalescent sera*
	Days after onset of illness				
	0–3	4–8	>8	Total	
Positive (%)	9 (50%)	12 (44%)	0	21 (40%)	0
Negative	9	15	8	32	21
Total	18	27	8	53	21

*PCR test was performed only in convalescent sera of patients who tested positive in acute sera for VZV DNA

Table 2

IgM antibodies against herpes simplex virus (HSV) and Epstein-Barr virus (EBV) in acute and convalescent sera of 53 patients with herpes zoster who tested positive for VZV IgM.

Material	VZV IgM EIA (Virotech, Germany) positive	HSV IgM EIA (Virotech, Germany)*		EBV VCA IgM IFA Institute Virion, (Switzerland)*	
		Positive	Negative	Positive	Negative
Acute sera	5	0	5	0	5
Convalescent sera	20	0	20	0	20

*HSV IgM EIA and EBV capsid antigen IgM IFA were performed in all patients who tested VZV IgM positive

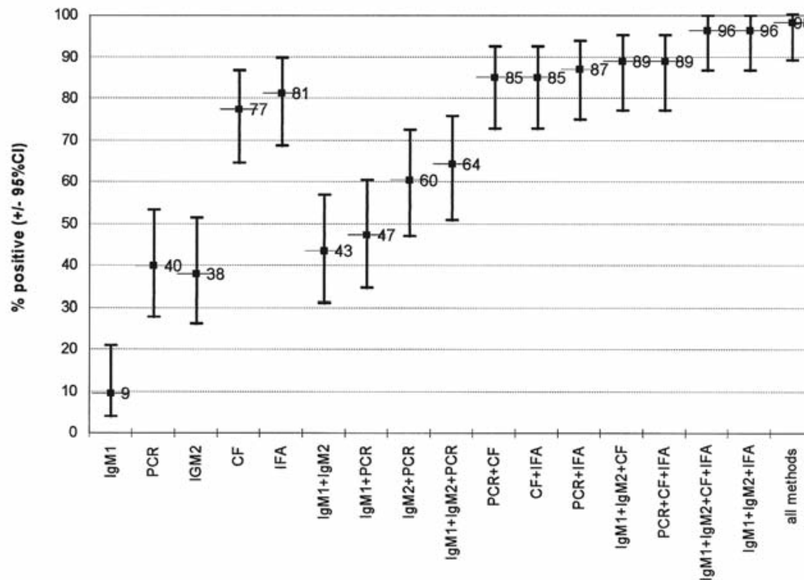

Figure 2

Comparison of the performance of four diagnostic methods and their combinations in acute and convalescent sera of 53 patients with herpes zoster. IgM1 and IgM2 = enzyme linked immunosorbent assay (EIA) for varicella-zoster virus (VZV) IgM antibody detection from acute and convalescent serum respectively; PCR = real-time polymerase chain reaction for the presence of VZV DNA in acute serum; CF = complement fixation test for complement fixing VZV antibody detection in paired sera; IFA = indirect fluorescent antibody test for VZV IgG antibody detection in paired sera. The results are shown as proportions of positive testing with their 95% confidence intervals (modified Wald, based on Agresti and Coull, 1998).

Discussion

Patient serum was used in the present study as starting material for simultaneous determination of antibody response to VZV together with VZV load in the paired sera of otherwise healthy adult patients with acute *herpes zoster* in the course of clinical illness; no atypical zoster cases were included.

Surprisingly low prevalence of VZV IgM antibodies (9%) was detected by the EIA in the acute *zoster* sera and the majority of *zoster* patients (38%) also remained IgM negative in the convalescent sera. The frequency of IgM antibodies in the *zoster* patients described by other authors varied widely from 10 to over 70% depending on the method used, the patients' clinical status and the time of specimen collection [10–12, 18]. However, even with the most sensitive test system IgM responses were not detected in a satisfactory proportion of patients and the IgM assay is not a particularly helpful approach to *zoster* serodiagnosis [11, 19].

In contrast to other studies, we observed no IgM cross-reactivity between VZV and HSV in the sera of *zoster* patients who were VZV IgM positive [10]. IgM cross-reactivity between VZV and HSV appears to depend on the test system used and is seen in acute VZV infection rather than during reactivation. This finding is of definite interest since it is a frequently observed clinical and laboratory problem.

No serological cross-reactivity with EBV was noted.

The combination of CF and IFA gave positive results in 45 out of 53 (85%) paired sera tested and the combination of all serological methods identified 51 (96%). The combination of IgM, CF and IFA proved to be the most sensitive approach for the laboratory diagnosis of VZV reactivation by serology in this study.

Although CF and IFA tests showed very high sensitivity, they are of limited value for early diagnosis of zoster when therapy can be initiated and only a retrospective serological confirmation of zoster after analysis of paired serum samples is possible. Nevertheless, serodiagnosis of VZV reactivation remains useful in confirming the clinical diagnosis, particularly when vesicle specimens are not available.

To improve diagnostic sensitivity for the acute serum samples, real-time PCR was performed on all acute *zoster* sera and convalescent sera of those patients who tested initially positive for VZV DNA by PCR.

In the present study VZV-DNA was detected in 21 (47%) of 45 samples collected within the first 8 days of illness and in 21 of the total of 53 acute zoster sera (40%). It was no longer detectable in the convalescent sera of patients who tested initially positive for VZV DNA in the acute sera. Our results show that VZV viraemia in zoster patients lasts only days in serum. This finding is comparable to the results reported by other authors, although the sensitivity of our PCR test was lower compared to other studies [10, 14]. To increase the sensitivity of PCR different strategies may be employed, such as increasing the volume of plasma for DNA extraction, reducing the volume of elution buffer, spinning down virus particles in the plasma prior to extraction or switching the target sequence from a unique sequence such as the gI glycoprotein gene (late gene 67) sequence exploited in this study to repetitive sequences in the VZV genome [20]. In a previous study, we demonstrated the beneficial impact of higher plasma volumes used for VZV DNA extraction on the sensitivity of the PCR assay [15]. Other studies described primers amplifying DNA fragments from other VZV genes and primers se-

lected from VZV genes 28 (early gene, encoding DNA polymerase) and 29 (early gene, encoding single stranded DNA binding protein) proved to be highly sensitive [10, 14, 20].

In summary, VZV DNA can be detected by PCR in serum and not only from vesicle fluid. Hence both serology and VZV load from the same serum sample are possible and can be used for routine purposes, particularly when vesicle specimens are not available. Serum DNA detection can be used for early diagnosis of VZV reactivation if patient samples are collected within first days of illness.

IgM antibodies in the acute patient serum are not reliable markers for the diagnosis of herpes zoster and even in the convalescent serum only 38% of samples gave a positive reaction. IgM results must therefore be interpreted with caution.

If the PCR and IgM tests on the acute serum are negative, VZV reactivation is not ruled out and serology can be performed on the convalescent serum.

Optimal laboratory sensitivity in the diagnosis of herpes zoster from serum can be achieved by the combination of PCR and serology of paired serum samples.

Serological methods are of limited value for early diagnosis of zoster when therapy can be initiated, because CF and IFA need convalescent serum and IgM test sensitivity is insufficient.

Early diagnosis of VZV reactivation by PCR is possible from serum in the first days of illness and test sensitivity requires further improvement.

The findings highlight the need for future studies investigating the usefulness of PCR and serology in atypical cases of VZV reactivation without skin eruptions and with an unusual clinical presentation.

The authors thank Dr. Meinrad Good (Target BioScience AG) for his support in statistical analysis and for valuable discussions.

Correspondence:
Marinko Dobec, MD, PhD
medica,
Medizinische Laboratorien Dr. F. Kaeppeli
Wolfbachstrasse 17
CH-8024 Zurich
Switzerland
E-Mail: m.dobec@medica-labor.ch

References

1 Arvin AM. Varicella-zoster virus. Clin Microbiol Rev. 1996;9:361–81.
2 Gruninger T, Dobec M, Keller A. Asymptomatic varicella infections in pregnancy: detection and problems. Gynäkol Geburtshilfliche Rundsch. 1994;34:171–4.
3 Schunemann S, Mainka C, Wolff MH. Subclinical reactivation of varicella-zoster virus in immunocompromised and immunocompetent individuals. Intervirology. 1998;41:98–102.
4 Mehta SK, Cohrs RJ, Forghani B, Zerbe G, Gilden DH, Pierson DL. Stress-induced subclinical reactivation of varicella zoster virus in astronauts. J Med Virol. 2004;72:174–9.
5 Sweeney CJ, Gilden DH. Ramsay Hunt syndrome. J Neurol Neurosurg Psychiatry. 2005;71:149–54.
6 Morita Y, Osaki Y, Doi Y, Forgani B, Gilden DH. Chronic active VZV infection manifesting as zoster sine herpete, zoster paresis and myelopathy. J Neurol Sci. 2003;212:7–9.
7 Furuta Y, Ohtani F, Kawabata H, Fukuda S, Bergstrom T. High prevalence of varicella-zoster virus reactivation in herpes simplex virus-seronegative patients with acute peripheral facial palsy. Clin Infect Dis. 2000;30:529–33.
8 de Jong MD, Weel JF, van Oers MH, Boom R, Wertheim-van Dillen PM. Molecular diagnosis of visceral herpes zoster. Lancet. 2001;357:2101–2.
9 Furuta Y, Ohtani F, Sawa H, Fukuda S, Inuyama Y. Quantitation of varicella-zoster virus DNA in patients with Ramsay Hunt syndrome and zoster sine herpete. J Clin Microbiol. 2001;39:2856–9.
10 Sauerbrei A, Eichhorn U, Schacke M, Wutzler P. Laboratory diagnosis of herpes zoster. J Clin Virol. 1999;14:31–6.
11 Schmidt NJ, Arvin AM. Sensitivity of different assay systems for immunoglobulin M responses to varicella-zoster virus in reactivated infections (zoster). J Clin Microbiol. 1986;23:978–9.
12 Gershon AA, Steinberg SP, Borkowsky W, Lennette D, Lennette E. IgM to varicella-zoster virus: demonstration in patients with and without clinical zoster. Pediatr Infect Dis. 1982;1:164–7.
13 Kuhn JE, Klaffke K, Munk K, Braun RW. HSV-1 gB and VZV gp-II crossreactive antibodies in human sera. Arch Virol. 1990;112:203–13.
14 de Jong MD, Weel JF, Schuurman T, Wertheim-van Dillen PM, Boom R. Quantitation of varicella-zoster virus DNA in whole blood, plasma, and serum by PCR and electrochemiluminescence. J Clin Microbiol. 2000;38:2568–73.
15 Kronenberg A, Bossart W, Wuthrich RP, Cao C, Lautenschlager S, Wiegand ND, et al. Retrospective analysis of varicella zoster virus (VZV) copy DNA numbers in plasma of immunocompetent patients with herpes zoster, of immunocompromised patients with disseminated VZV disease, and of asymptomatic solid organ transplant recipients. Transpl Infect Dis. 2005;7:116–21.
16 Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. Amer Statistician. 1998;52:119–26.
17 Aizawa H, Ohtani F, Furuta Y, Sawa H, Fukuda S. Variable patterns of varicella-zoster virus reactivation in Ramsay Hunt syndrome. J Med Virol. 2004;74:355–60.
18 Terada K, Niizuma T, Kawano S, Kataoka N, Akisada T, Orita Y. Detection of varicella-zoster virus DNA in peripheral mononuclear cells from patients with Ramsay Hunt syndrome or zoster sine herpete. J Med Virol. 1998;56:359–63.
19 Oladepo DK, Klapper PE, Percival D, Valley PJ. Serological diagnosis of varicella-zoster virus in sera with antibody-capture enzyme-linked immunosorbent assay of IgM. J Virol Methods. 2000;84:169–73.
20 Quinlivan M, Breuer J. Molecular studies of varicella zoster virus. Rev Med Virol. 2006;16:225–50.

SMW

Established in 1871

Formerly: Schweizerische Medizinische Wochenschrift

Swiss Medical Weekly

The European Journal of Medical Sciences

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising. The 2006 impact factor is 1.346.
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of professional statisticians for every article with statistical analyses

- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing

Editorial Board

Prof. Jean-Michel Dayer, Geneva
Prof Paul Erne, Lucerne
Prof. Peter Gehr, Berne
Prof. André P. Perruchoud, Basel
Prof. Andreas Schaffner, Zurich (editor in chief)
Prof. Werner Straub, Berne (senior editor)
Prof. Ludwig von Segesser, Lausanne

International Advisory Committee

Prof. K. E. Juhani Airaksinen, Turku, Finland
Prof. Anthony Bayes de Luna, Barcelona, Spain
Prof. Hubert E. Blum, Freiburg, Germany
Prof. Walter E. Haefeli, Heidelberg, Germany
Prof. Nino Kuenzli, Los Angeles, USA
Prof. René Lutter, Amsterdam, The Netherlands
Prof. Claude Martin, Marseille, France
Prof. Josef Patsch, Innsbruck, Austria
Prof. Luigi Tavazzi, Pavia, Italy

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts:	submission@smw.ch
Letters to the editor:	letters@smw.ch
Editorial Board:	red@smw.ch
Internet:	http://www.smw.ch

EMH FMH Schwabe
Editores Medicorum Helveticorum

Official journal of the Swiss Society of Infectious Diseases, the Swiss Society of Internal Medicine and the Swiss Respiratory Society

Supported by the FMH (Swiss Medical Association) and by Schwabe AG, the long-established scientific publishing house founded in 1488