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Editorial

We would like to inform our readers that Association of Basic Medical Sciences, beginning with this year, will publish Bosnian Journal of Basic Medical Sciences at least quarterly, depending on your submissions.

As it is previously announced, the second opening lecture form of the International and Scientific Symposium about human embryo and its cloning held in Sarajevo, 15 February 2003 ("Scientific, ethical and religious dilemmas over the status of human embryo and its cloning") is published in this issue.

In this issue, in concordance with the current world situation and after the suggestions of some of our distinguish colleagues, we decide to publish a review article on aetiology and epidemiology of Severe Acute Respiratory Syndrome (SARS) - a new emerging disease.

We kindly remind our readers to inform other colleagues and associates about the Association and our membership.

All observation, suggestions, and proposals from our readers are more than welcome and will improve the quality of the Journal.

We are inviting scientists from not only entire Bosnia and Herzegovina but internationally, to participate in next issues and hope that many of them will answer our invitation.

Sarajevo, May, 2003

Editorial Board
Prologue

“We think that science has already explained all when explained movement of the Moon around the Earth. But the real world is not universe like a simple pendulum clock.”

Jim York, a physicist from the University of Maryland who coined the name "chaos"

We are assured that the field of "Human Cloning" comprise the most crucial scientific questions of present time. It seems that all exaltation of its progress and fascinating results have vanished while being replaced with the cogitative concern that Immanuel Kant expressed in three fundamental questions:

What can I know?
What should I do?
What may I hope?

While considering all these questions famous philosopher thought about any human being.

Today, these queries consideration is based on the experience of illuminator "ingress" in all that exist, as well as in the human being itself. Experience of the "illumination" triumph seduces science beyond its achievements and freedoms. We would say that science wants even beyond its real wants. Nowadays, global scientific impact appears as general opinions correlated to the universe of technological utilisation, professionalism in knowledge and biological science influence on the behavioural inducements.

The basic thinking principles established by modern science are inseparable from the statements of philosopher Descartes: "World of mind and world of body are separated as individual substances that exist separately without any needful co-existence". In that way, a new authoritative scientific relation towards the world has been created, out of which, the field of values "Heavens are devoid of the glory of God" (A.N. Whitehead, "Science and the modern world") has been completely excluded or eliminated.

It is obvious that the crucial postulations of nature are created throughout gathering of the sufficient data and simplification of the numerous correlative causes and consequences in other to elucidate "the anarchy of systems" and accomplish the exact predictions of their attitudes. A amazing technological progress in 20th century made many people believe that some day science would find out all ignorance of nature and improve the control over it. According to that assumption, the attitude of very complex dynamic systems would finally conform to the scientific formulations and calculations.

A fascinating thoughtful help of chaosologists, particularly in their theory of fractals that revealed an apprehension of the reality as made from worlds within self-similar worlds i.e. worlds within dimensions, is necessary for the proper quantification of defect associated with the progress in knowledge of life. That knowledge has been previously neglected to the simple scientific facts and responsibilities (especially in medicine) that naturally appear from inside when life is recognised as a holistic system.

About cloning

During the last 5 years, human cloning has become a field of particular public interests, attentions and serious moral discussions. From February 1997, when the news about the first successful mammal cloning (sheep Dolly) was resounded, till present time several mammal species have been cloned (Dolly, the world's first cloned sheep, has been euthanized after being diagnosed with progressive lung disease) (Table 1).

Although human child cloning is still being uncertain and animal experiments are demonstrating low success rate, the production of the functional mammal clones is indicating real possibilities of the human cloning process. In November 2001, American researchers announced that they produced the first cloned human embryos despite the fact that study had been carried out on only six cells and embryos did not survive. Additionally, a few specialists in fertilisation proclaimed their intention to perform cloning of human beings.

Correct and reliable terminology

Today, it is recognized that there is no agreement about the terms used to discuss human cloning, regarding both the activities involved and the entities that result. The terminology ever used should image descriptive reality of
the substance in the most reliable manner in order to submit the moral arguments to the relevant authorities. The proper terminology should overcome artificial remoulding in moral question resolving or denying of the crucial moral elements in the terms with obvious facing the moral enquiries.

According to the studious analysis of cloning activity and its correlations to the accomplished meanings and purposes, as well as according to the extensive critical analysis of the alternative terminology, the following definitions might be adopted as core terms when discussing the subject of human cloning:

- Cloning: A form of reproduction in which offspring result not from the chance union of egg and sperm (sexual reproduction) but from the deliberate replication of the genetic makeup of another single individual (asexual reproduction).
- Human cloning: The asexual production of a new human organism that is, at all stages of development, genetically virtually identical to a current existing or previously existing human being. It would be accomplished by introducing the nuclear material of a human somatic cell (donor) into an oocyte (egg) whose own nucleus has been removed or inactivated, yielding a product that has a human genetic constitution virtually identical to the donor of the somatic cell (this procedure is known as "somatic cell nuclear transfer").

Instead of use the terms "reproductive cloning" and "therapeutic cloning" the following designations should be used:

- Cloning to produce children: Production of a cloned human embryo, formed for the proximate purpose of initiating a pregnancy, with the ultimate goal of producing a child who will be genetically virtually identical to a currently existing or previously existing individual.

<table>
<thead>
<tr>
<th>ANIMAL SPECIES</th>
<th>DONOR CELL</th>
<th>NUMBER OF TRANSFERR ED CLONED EMBRYOS</th>
<th>NUMBER OF BORN-ALIVE INDIVIDUALS</th>
<th>PERCENTAGE OF BORN-ALIVE INDIVIDUALS PER TRANSFERRED EMBRYOS</th>
<th>REFERENCES</th>
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<tr>
<td>Sheep</td>
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<td>Bovine</td>
<td>Foetal Fibroblasts, Cumulus and Tuba Uterina Epithelial Cells</td>
<td>496</td>
<td>24-30#</td>
<td>4.8-6%</td>
<td>2a</td>
</tr>
<tr>
<td>Mouse</td>
<td>Cumulus Cells                                                                 2468</td>
<td>31**</td>
<td>1.3%</td>
<td>3</td>
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<tr>
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<td>Transgenic Foetal Fibroblasts                                                97</td>
<td>5</td>
<td>5.2%</td>
<td>4a</td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>Foetal Fibroblasts                                                            110</td>
<td>1</td>
<td>0.9%</td>
<td>5a</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
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<tr>
<td>Rabbit</td>
<td>Cumulus Cells                                                                 371</td>
<td>6</td>
<td>1.6%</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

References:

Table 1 Comparative data on born-alive cloned animals (NAS Report on Scientific and Medical Aspects of Human Cloning)
- Cloning for biomedical research: Production of a cloned human embryo, formed for the proximate purpose of using it in research or for extracting its stem cells, with the ultimate goal of gaining scientific knowledge of normal and abnormal development and of developing cures for human diseases.

- Cloned human embryo: A human embryo resulting from the nuclear transfer process (as contrasted with a human embryo arising from the union of egg and sperm), the immediate (and developing) product of the initial act of cloning, accomplished by successful somatic cell nuclear transfer, whether used subsequently in attempts to produce children or in biomedical research.

**Ethical and legal consideration of the merits of cloning-to-produce-children**

**Ethics of cloning-to-produce-children**

The prospect of cloning-to-produce children raises a host of moral questions, among them following are the most important:

1. Could the first attempts to clone a human child be made without violating accepted moral norms governing experimentation on human subjects?
2. What harms might be inflicted on the cloned child as a consequence of having been made a clone?
3. Is it significant that the cloned child would inherit a genetic identity lived in advance by another and, in some cases, the genetic identity of the cloned child's rearing parent?
4. How might cloning-to-produce-children affect relationships within the cloning families? More generally, how might it affect the relationship between the generations?
5. How might it affect the way society comes to view children?
6. Other questions

A broad ethical evaluation of all above mentioned problems regarding the value of cloning-to-produce children is an obligation. Two reports by the National Bioethics Advisory Commission of the United States of America, 1997 and 2002, concluded that attempts to clone a human being would be unethical "at this time" due to safety concerns and the likelihood of harm to those involved.

National Academy of Sciences of the United States of America alleges:

“Our present opposition to human reproductive cloning is based on science and medicine, irrespective of broader considerations. We stress, however, that a broad ethical debate must be encouraged so that the public can be prepared to make decisions if human reproductive cloning is some day considered medically safe for mothers and offspring”.

**Purposes**

In recent years, in anticipation of cloning-to-produce-children, proponents have harmonised a variety of possible uses of this technology. The desire to control or select the genomes has been observed in more than a few prospective users around the world.

Although we appreciate that a perfected technology, once introduced for one purpose, might then be used for any other purpose, we shall state only purposes that seem to us to merit serious consideration.

1. **Production of Biologically Related Children**

Human cloning would allow individuals or couples with fertility problems to have biologically related children. In addition, it would allow married couples with fertility problems to avoid using donor gametes, and therefore avoid raising children with genetic inheritances from outside the marriage.

2. **Avoidance of Genetic Diseases**

Human cloning could allow couples at risk of generating children with genetic disease to have healthy children (for example, if both parents carry one copy of a recessive gene for the same hereditary disorder).

3. **Production of "Rejection-Proof" Transplants**

Human cloning could produce ideal transplant donors for people who are sick or dying. Cloning could potentially serve the human goods of beginning a new life and saving an existing one.

4. **"Replication" of a Loved One**

Human cloning would allow parents to "replicate" a dead or dying child or relative.

5. **Reproduction of Individuals of Great Genius, Talent, or Beauty**

Human cloning would allow families or society to reproduce individuals of great genius, talent, or beauty, where these traits are presumed to be based on the individuals' desirable or superior genetic make-ups.
Arguments for cloning-to-produce children

The purposes or reasons for cloning-to-produce-children are clearly intelligible and stated. When challenged, the defenders of these purposes often appeal to the larger moral and political goods. These typically fall within the following three categories: human freedom, existence, and well-being.

1. The Goodness of Human Freedom

Strictly speaking, the appeal to human freedom is not so much a defence of cloning itself as it is of the right to practice it, asserted against those who seek to prohibit it. In Eisenstadt v. Baird (1972), the United States Supreme Court enunciated the principles of reproductive freedom: "If the right to privacy means anything, it is the right of the individual, married or single, to be free from unwarranted intrusion and interference into matters so affecting a person as a decision whether to bear or beget a child."

2. The Goodness of Existence

Like the appeal to freedom, the appeal to the goodness of existence is not an argument for cloning, but an argument against opponents who speak up in the name of protecting the cloned child against the harms connected with its risky and strange origins as a clone.

3. The Goodness of Well-Being

The third moral argument for cloning-to-produce-children is that it would contribute in certain cases to the fulfilment of human goods that are widely honoured and deeply rooted in modern democratic societies.

Arguments against cloning-to-produce children

The Ethics of Human Experimentation

We may begin with concerns regarding the safety of the cloning procedure and the health of the participants. If carefully considered, these concerns begin to image the important ethical principles that must guide our broader assessment of cloning-to-produce-children. It is obvious that human beings, unlike inanimate matter or even animals, are in some way inviolable, and therefore challenge us to reflect on what it is about human beings that makes them inviolable, and whether cloning-to-produce-children threatens these distinctly human goods.

1. Problems of Safety

Cloning-to-produce-children is not now safe. Even most proponents of cloning-to-produce-children generally qualify their support with a caveat about the safety of the procedure. Safety concerns revolve around potential dangers to the cloned child, as well as to the egg donor and the woman who would carry the cloned child to birth.

2. Risks to the child

Risks to the cloned child-to-be must be taken especially seriously, both because they are most numerous and most serious and because, unlike the risks to the egg donor and birth mother, they cannot be accepted knowingly and freely by the person who will bear them.

3. Risks to egg donor and birth mother

These include risks to the future reproductive health caused by the hormonal treatments required for egg retrieval and general health risks resulting from the necessary superovulation.

Animal studies suggest the health risks to the woman who carries the cloned foetus to term. The late-term foetal losses and spontaneous abortions occur substantially more often with cloned foetuses than in natural pregnancies. In humans, such late-term foetal losses may lead to substantially increased maternal morbidity and mortality. In addition, many pregnancies involving cloned foetuses result in serious complications.

Reflecting on the mentioned dangers the National Academy of Sciences of the United States of America concluded:

"Results of animal studies suggest that reproductive cloning of humans would similarly pose a high risk to the health of both foetus or infant and mother and lead to associated psychological risks for the mother as a consequence of late spontaneous abortions or the birth of a stillborn child or a child with severe health problems".

Moral concern

Because of these risks, there is widespread agreement that, at least for now, attempts at cloning-to-produce-children constitute unethical experimentations on human individuals and are therefore impermissible. National Academy of Sciences, in January 2002, recommended that the United States should ban such cloning for at least five years.

These questions of the ethics of research, particularly the issue of physical safety, point clearly to the conclusion that cloning-to-produce-children is unacceptable and should not be attempted.
The Ethics of Cloning-for-Biomedical-Research

The Manner and Spirit of This Inquiry

The question of whether or not to proceed with human cloning-for-biomedical-research is a morally serious and difficult one. On the one hand, there is the promise that such research could lead to important knowledge of human embryological development and gene action, especially in cases in which there are genetic abnormalities that lead to disease.

There is also the promise that such research could contribute to producing transplantable tissues and organs that could be effective in curing or reversing many dreaded illnesses and injuries. On the other hand, there are the morally relevant facts that this research involves the deliberate production, use, and ultimate destruction of cloned human embryos, and that the cloned embryos produced for research are no different from cloned embryos that could be used in attempts to produce cloned children.

The Nuremberg Code, the Helsinki Declaration, and the Belmont Report are all efforts to set moral limits on biomedical research and to ensure that science serves human beings rather than the other way around. Among other things, these ethical caudexes embody the recognition that those who do research about human beings can never escape, nor should they, their status as human beings. Those who investigate human biology are always both the knower and the subject that is known, both the potential healers and the potentially afflicted. And therefore they must never treat that which is their equal, their fellow human beings, as something less than human.

Arguments for Cloning-for-Biomedical-Research

The moral arguments for cloning-for-biomedical-research can be stated in the following straightforward way: Modern and human communities in general have an obligation to try to heal the sick and relieve their suffering. This obligation, deeply rooted in the moral teaching of "love of neighbour," lies heaviest on physicians and health-care professionals who attend to individual patients. But it guides also the activities of biomedical scientists and biotechnologists whose pioneering research and discoveries provide new and better means of healing and relieving those who suffer. Research on cloned human embryos is one more path to discovering such means.

1. Cloning to Improve Understanding of Human Disease

The creation of cloned embryos using nuclei from individuals carrying genetic mutations, specifically genes that predispose them to particular diseases, might be used to better understand and treat those diseases.

2. Cloning to Devise New Treatments for Human Diseases

The same cellular model systems used to study disease processes are also potentially useful for assessing and developing chemical or pharmaceutical treatments for some diseases.

3. Cloning to Produce Immune-Compatible Tissues for Transplantation

Some animal studies suggest that tissues derived from embryonic stem cells can, if injected under certain conditions, populate disease-stricken areas and differentiate so as to compensate for the loss of function caused by the diseased tissue. Cloning-for-biomedical-research offers the possibility to generate individualized, "rejection-proof" replacement cells and tissues to help patients fight disease.

4. Cloning to Assist in Gene Therapy

Cloning techniques could also be combined with precise genetic manipulation to devise genetic treatments for genetic diseases. For example, a cloned embryo produced from a patient with severe combined immunodeficiency could be genetically modified to correct the disease-causing mutation.

Possible Moral Dilemmas of Proceeding

Yet the moral dilemmas of proceeding, still to be considered, are the subject of some debate among us. There are two different positions these are principal moral aspects for cloning for biomedical research.

Position Number One

Moral controversy is that it involves the production, use, and intentional destruction of cloned human embryos. To determine whether or not the science should proceed, or
if it does, what limits should be placed on this research, it must be asked what is owed this nascent form of human life.

Position Number Two

Where to set the boundary for the embryo utilisation is a matter for prudent judgment. For the foreseeable future, the moral line might be safely drawn at fourteen days of development, when no nervous system has developed and when a distinct identity as a single individual has not yet been preordained.

Arguments against Cloning-for-Biomedical-Research

The case for treating the early-stage embryo, as simply the moral equivalent of all other human cells, is entirely unconvincing: it denies the continuous history of human individuals from zygote to foetus to infant to child. It misunderstands the meaning of potentiality and, specifically, the difference between a "being-on-the-way" (such as a developing human embryo) and a "pile of raw materials," which has no definite potential and which might become anything at all; and it ignores the hazardous moral precedent that the routinized creation, use, and destruction of nascent human life would establish for other areas of scientific research and social life.

It is not possible to be persuaded by the argument that fourteen days marks a significant difference in moral status. Embryo's human and individual genetic identity is present from the start; nothing that happens later during the continuous development that follows, at fourteen days or any other time, is responsible for appearance of a novel human individuality or identity.

1. Asexual Reproduction and the Genetic Manipulation of Embryos

Cloning-for-biomedical-research and cloning-to-produce-children both begin with the same act of cloning: the production of a human embryo that is genetically virtually identical to its progenitor. But we should not forget the agreement at the start to clone: saying yes to cloned embryos in laboratories means saying yes in principle to genetic masteries of one generation over the next.

2. The Complete Instrumentalization of Nascent Human Life

By approving the production of cloned embryos for the sole purpose of research, society would meet yet another moral boundary: separating the different ways in which embryos might become available for human experimentation. In the eyes of those who create in vitro fertilisation embryos to produce a child, every embryo, at the moment of its creation, is a potential child.

3. Opening the Door to Other Moral Hazards

This leads directly to our third concern— that the cloning of human embryos for research will open the door to additional, maybe even greater, moral hazards. Human suffering from horrible diseases never comes to an end, and likewise, our willingness to use embryonic life in the cause of research, once permitted, and is also unlikely to find any natural stopping point.

In addition, the reasons justifying production of cloned embryos for research can be predicted to expand. Today, the demand is for stem cells; tomorrow it may be for embryonic and foetal organs.

Epilogue

Simplifying of nature to the several measurable "principles" is an opinion ambient of contemporary science in which medicine finds its own place. It seems that such position has been seriously unsettled by the theory of chaosologists (scientists preoccupied with the chaos theory) about chaotic dynamic system. That system is extremely sensitive because it is always in motion, it is ever changeable, and it never entirely returns to its primary state.

We cite:

"It is like a variable time river", according to Heraclites who said: "All things go and nothing stays and you could not step twice into the same river". It is moreover correct for the real river and crucial for the chaos. It is obvious that, when even more complex dynamic system act regularly at some level, a "sensitive" principle of chaos might affect it by subtle process of sequestering and fracturing.

For example, even identical twins sharing the same DNA will often turn out quite differently because the DNA molecule will take a slightly different course in the development of each child. Development of embryo is dynamic system and its extreme susceptibility to the primary conditions creates innate chaos to ensure that 'identical' twins are never perfectly identical. But we should become opened for the different abundance of knowledge arising from the culture of the world control: "This century science reveals a desire of nature to remain hidden and out of our comprehension" (Fractals, John Briggs)
References


Therapeutic effects of two antidepressant agents in the treatment of posttraumatic stress disorder (PTSD)

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Abstract

Posttraumatic stress disorder (PTSD) is a psychiatric disorder characterised by an acute emotional response to a traumatic event or situation involving severe environmental stress (natural disasters, wars, epidemics, rape, assaults, physical torture, catastrophic illness or accident), which may be identified in cognitive, affective or sensory motor activities. The objective was to perform a pilot clinical trial designed to compare the effects of older (tricyclic) and newer “second-generation” (selective inhibitors of serotonin uptake) antidepressants in the treatment of PTSD. A total of 20 hospitalised chronic military combat Bosnian veterans with PTSD symptoms were randomly assigned into two groups of 10 patients each. One group was treated with amitriptyline hydrochloride (AMYZOL®) 75 mg/day as a representative of older antidepressants and the other with fluoxetine hydrochloride 60 mg/day (OXETIN®) as a representative of newer antidepressants. Those drugs were administered by mouth two or three times-a-day in equally divided doses for at least 8 weeks. Favourable response was achieved in 70% of patients treated with amitriptyline hydrochloride and 60% of patients treated with fluoxetine hydrochloride. Amitriptyline hydrochloride was more effective in the treatment of acute PTSD symptoms (emotional numbing, startle reaction, nightmares, flashbacks, intrusive thoughts, vulnerability, poor impulse control or irritability and explosiveness). Fluoxetine hydrochloride showed a greater efficacy in the treatment of chronic PTSD symptoms (avoidance and numbing symptoms, hyperarousal, nightmares and a feeling of guilt).

Key words: Posttraumatic stress disorder, amitriptyline, fluoxetine, PTSD total scores

Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric disorder characterised by an acute emotional response to a traumatic event or situation involving severe environmental stress (natural disasters, wars, epidemics, rape, assaults, physical torture, catastrophic illness or accident), which may be identified in cognitive, affective or sensory motor activities (DSM-III, 1980). It has been reported that prevalence of PTSD among Vietnam veterans was approximately 67% (YEHYDA, 1999), in people experiencing natural disasters or catastrophes 30% (YEHYDA, 1999), and in women experiencing sexual abuse or sexual assault (rape) between 57% and 80% (REGEHR, 1999).

According to DSM-III criteria the precipitating event in PTSD should be "outside of range of usual human experience." However, there is evidence that a PTSD-like syndrome can occur following more usual life traumas (e.g. bereavement) (HOROWITZ et al., 1980). Using an experimental approach, BLANCHARD et al. (1982) have reported that heart rate response to audiotape of combat sounds successfully differentiated normal from PTSD patients in 95.5% of cases. Systolic blood pressure and forehead electromyographic response also differed between groups.

In DSM-IV (1994) are described three subtypes of PTSD:

- Acute (duration of symptoms less than 3 months)
- Delayed (onset at least 6 months after trauma) and
- Chronic (duration of symptoms 3 or more than 3 months).

Data sources (DAVIDSON et al., 1985; GREEN et al., 1985; SIERLES et al., 1983) indicate that there is a high prevalence (75-84%) of concurrent psychiatric diagnoses in PTSD patients. The most common disorders found were alcohol or drug abuse (60%), depression (20%), generalised anxiety disorders (14%) and antisocial personality (11%).

Objective

Since pharmacotherapy of PTSD may include the administration of antidepressant agents, the objective of this study was to perform a pilot clinical trial designed to compare the effects of the representatives of older and newer antidepressants in the treatment of PTSD.
Methods
(patient and trial characteristics)

Type of trial
A pilot randomised single blind trial.

Patient selection
A total of 20 hospitalised chronic military combat Bosnian male veterans between 25-50 years of age.

PTSD assessment
- DSM-IV (1994) and ICD-X (1994) diagnostic criteria
- Standard Psychiatric Interview (SPI)

Inclusion criteria
- Only DSM-IV diagnostic criteria proven PTSD
- Only ICD-X diagnostic criteria proven PTSD
- Only HTQ diagnostic criteria proven PTSD
- Only SPI diagnostic criteria proven PTSD

Exclusion criteria
- History of alcohol and/or drug abuse
- History of depression
- History of generalised anxiety
- History of personality disorder (antisocial personality)

Drug administration
Patients with proven PTSD were randomly assigned into two groups. Each group of 10 patients was treated with one of two investigated antidepressants, which were administered by mouth two or three times-a-day in equal divided doses for at least 8 weeks:

- One group was treated with amitriptyline hydrochloride (AMY ZOL®) 75 mg/day t.i.d.
- The other group was treated with fluoxetine hydrochloride (OXETIN®) 60 mg/day b.i.d.

Results
Favourable response was achieved in 70% of patients treated with amitriptyline hydrochloride and 60% of patients treated with fluoxetine hydrochloride.

Amitriptyline hydrochloride produced a marked decrease of emotional numbing and other acute PTSD symptoms including startle reaction, nightmares, flashbacks, intrusive thoughts, vulnerability, poor impulse control or irritability and explosiveness.

Fluoxetine hydrochloride showed a greater efficacy in the treatment of chronic PTSD symptoms (avoidance and numbing symptoms, hyperarousal, nightmares and a feeling of guilt).

PTSD total scores before and after administration of amitriptyline hydrochloride and fluoxetine hydrochloride are shown in Figs. 1, 2, 3 and 4.

Discussion
Treatment of PTSD consists of behaviour therapy, pharmacotherapy, and psychotherapy. The pharmacotherapy of PTSD is not well established. Broad treatment guidelines are not curative, but instead are directed at ameliorating PTSD symptomatology. The positive symptoms, including re-experiencing the event and hyperarousal, often respond to pharmacotherapy. Negative symptoms,
such as avoidance and withdrawal, are usually resistant to medication. Due to the overlapping symptoms between PTSD and other psychological disorders, pharmacological treatment usually involves antidepressants (BLEICH, 1986).

Since pharmacotherapy of PTSD may include the administration of antidepressant agents, the objective of this study was to perform a pilot clinical trial designed to compare the effects of the representatives of older and newer antidepressants in the treatment of PTSD.

Amitriptyline hydrochloride (AMYZOL®) and fluoxetine hydrochloride (OXETIN®) were representatives of older (tricyclic) and newer “second-generation” (selective inhibitors of serotonin uptake) antidepressants, respectively.

The mechanism of action of amitriptyline in man is thought to be inhibition of the membrane pump mechanism responsible for uptake of noradrenaline and serotonin in adrenergic and serotonergic neurons. This drug has very high ability to block serotonin uptake and moderate activity with respect to noradrenaline uptake. The diminution of monoamine oxidase (MAO) activity partially elucidates the antidepressant effect of amitriptyline (REYES and LISANSKY, 1984). Fluoxetine has been demonstrated to be a specific inhibitor of serotonin reuptake in vitro and in vivo in man and animals (LEMBERGER et al., 1978; LEMBERGER et al., 1978a; STARK et al., 1985).

The results of this pilot clinical trial are in agreement with other data sources. According to HTQ (1998), individuals with DSM-IV PTSD scores and/or total scores of > 2.5 are considered symptomatic for PTSD. This is the

**Figure 2.** PTSD total scores by age of patients after administration of amitriptyline hydrochloride (AMYZOL®) 75 mg/day p.o.

**Figure 3.** PTSD total scores by age of patients before administration of fluoxetine hydrochloride (OXETIN®) 60 mg/day p.o.
reason why PTSD total scores before and after administration of two antidepressant agents were scored. It has been found that before administration of both antidepressants all patients had PTSD total scores between 2.5 -5. At the end of treatment 70% of patients treated with amitriptyline hydrochloride and 60% of patients treated with fluoxetine hydrochloride had PTSD total scores < 2.5.

FALCON et al. (1985) have conducted uncontrolled clinical trial using amitriptyline hydrochloride and have reported that this drug had beneficial effects in treatment of PTSD in combat veterans.

In a double-blind study by DAVIDSON et al. (1990) that compared amitriptyline and placebo in 46 veterans with PTSD, modest benefits were reported. With an average daily dose of 169 milligrams, statistically significant improvement was seen after 4 weeks in the depression scale and after 8 weeks of treatment in the depression and anxiety scales. However, no significant improvements were noted on the intrusiveness scale.

CONNOR et al. (1999) have published that in a 12-week, double-blind study, fluoxetine (10 mg daily) was more effective than placebo for treating post-traumatic stress disorder (PTSD). It has been reported that on the Duke Global Rating (Duke) for PTSD, significantly more patients reached a score of 1 (no symptoms) during treatment with fluoxetine than placebo (59% versus 19%; p < 0.0005). The Davidson Trauma Scale (DTS) total scores were also significantly lower in patients treated with fluoxetine compared to placebo. The onset of beneficial effects was observed at 2 weeks on the Duke scale and at 4 weeks on the DTS. This study included only civilians, primarily women, who fulfilled DSM-IV criteria for PTSD.

Conclusions

A pilot clinical trial, designed to compare the effects of the representatives of older and newer antidepressants in the treatment of PTSD, was performed.

Two antidepressants used were:

- Amitriptyline hydrochloride (AMYZOL®) as a representative of older (tricyclic) antidepressants
- Fluoxetine hydrochloride (OXETIN®) as a representative of newer (selective inhibitors of serotonin uptake) antidepressants.

PTSD total scores before and after administration of amitriptyline hydrochloride and fluoxetine hydrochloride were assessed.

Of two antidepressants used, more favourable response was achieved with amitriptyline hydrochloride (70%) than with fluoxetine hydrochloride (60%).

Amitriptyline hydrochloride was more effective in the treatment of acute PTSD symptoms, while fluoxetine hydrochloride was more effective in the treatment of chronic PTSD symptoms.


Asymmetry of limbic structure (hippocampal formation and amygdaloidal complex) at PTSD

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Department of Anatomy, Faculty of Medicine, University of Sarajevo, ^ekaluša 90

Abstract
Defining exact position of weak anatomic function which is find in a base of neurological and psychiatric disorder is just became the subject of intensive research interest. For this purposes it is important to implement structural and functional MRI techniques, also for further lighten-ing and seeing subject of this work, more concretely connected to PTSD. Therefore, exactly MRI gives most sensitive volumetric measuring of hippocampal formation and amygdaloidal complex.
The goal of this work was to research asymmetry of hippocampal formation and amygdaloidal complex to the PTSD patients.
Results showed that at the axial slice length of hippocampal formation on the left and right side of all patients are significantly asymmetric. At the sagittal slice, there are no significant differences toward patient proportion according to symm. / asymm. of the hippocampal formation width at the right and left side. Difference in volume average of hippocampal formation between right and left side for axial and coronal slice is not statistically significant, but it is significant for sagittal slice. In about amygdaloidal complex patients with PTSD toward symm. / asymm. A mygdaloidal complex at the right and left side of axial and sagittal slice in all three measurement shows asymmetry, what is especially shown at sagittal slice. Difference in average length of amygdaloidal complex at the right and left side is not statistically significant for no one slice.

Therefore, results of a new research that are used MRI, showed smaller hippocampal level at PTSD (researched by Van der Kolka 1996, Pitman 1996, Bremner et al., 1995.). Application of MRI technique in research of asymmetry of hippocampal formation and amygdaloidal complex, which we used in our research, we recommend as a template for future researches in a sense of lighten-ing anatomic function that is a base of neuropsychiatric disorders.

Keywords: limbic system, hippocampal formation, amygdaloidal complex, asymmetry, posttraumatic stress disorder - PTSD

Introduction
Concerning development concept of PTSD since 1980 by American society of psychiatrics, officially is accepted what even laics knows, that extreme stress can lead to continuous psychiatric disorder. World health organization (WHO) PTSD entering in their 10 revision of International illness classification (IIK 10) and related health problems. Last years became clear and in specialist and scientific societies accepted that PTSD is not the only shape of psychopathological disorder that is caused by stress. Today it is find that in around 20% people who's passing through heavy stresses, heavy psychiatric disorders has been developed, or that in a around 18% of ambulance psychiatric patients psychiatric disorder for which they are contacting psychiatrics, are results of life trauma.

Hypersensibility, avoiding / emotional weakening, and repeatedly happening situations with hard remembrance of trauma are defined signs of PTSD. Authors presume that many of PTSD symptoms are result of limbic structure hyperactivity, which can through their many projections in to prefrontal cortex contribute to dysfunction of this system, and as results of that expect the loss in performance with frontal functioning. This hyperactivity can be physiological correlate of fear structure knowl-edge (hypothesis made by Foa, Feske, Murdock, Kozak I M McCarthy, 1991.), which are contagiously activated and can be leader of emotional abnormality, as sudden shows of hyper sensibility and sense of horror. Authors conclude that what ever neuropathological mechanism in sense is, no matter is this is prefrontal cortical pathology, lower function of limbic system, or most probably some combination of these two, their follows OR, OI and ONPSU damages in PTSD clearly contain involvement of frontal system.

Materials and Methods
As a material for construction of this study, we used 10 MRI scan patients with PTSP, where we have cognately function damage. Methods of work include measuring the size of hippocampal formations and amygdaloidal complex in all three projections (axial, coronal and sagittal) 10 patients with PTSP, where we have cognately functional damage.
MRI scans are done on MAGNET IMPACT SIEMENS 1.0 TESLA in T1 relaxation (TR 500 - 600 / TE 15 / field of view 180 x 260, the fatness layer SL 5 mm) and T2 relaxation (TR 4000 / TE 90 field of view 188 x 250 for axial and 173 x 230 for coronal, 210 x 240 for sagittal scans in 5 mm layer). Dual sequences are used PD and T2. In PD TR is 4000, and TE 22. We used a head - neck spiral, as well as a head spiral.

For the size measurement of amygdaloid complex and hippocampal formation, and their comparison from right to left, we used a program of evaluation - distance on the MRI from the Institute of Radiology of Clinical Centre in Sarajevo. We tested 10 patients with PTSP, approximate age 49.9 with standard deviation of 4.62 years.

The size of hippocampal formation is measurement in all three projections: horizontal (axial), frontal (coronal) and sagittal from right and left. The size of amygdaloid complex is measurement in two projections: horizontal (axial) and sagittal. In axial projections, we are measurement anterior - posterior and lateral - medial diameter of amygdaloid complex. We do not measurement the amygdaloid complex in coronal projections, because it is not possible his clear diffraction from the other cerebral structures. All values of hippocampal formation and amygdaloid complex are given in centimetres.

For hippocampal formation and amygdaloid complex in all three projections (axial, coronal and sagittal) are met in:

1. The number of patients with PTSD according to symmetry/asymmetry of hippocampal formation on the right and left side

2. A nalysis of patients with PTSD by the approximate size of hippocampal formation on left and right side Significant difference is tested with t-test

3. The number of patients with PTSD according to symmetry/asymmetry of amygdaloid complex on the right and left side on axial and sagittal projections

4. A analysis of patients with PTSD by the approximate size of amygdaloid complex on left and right side on axial and sagittal projections Significant difference is tested with t-test

Methods of statistical analysis used in this assignment are:

1. Arithmetic middle
2. Standard deviation
3. Standard failure
4. Median
5. Mod
6. Chi - Square test
7. t - test differences of arithmetical middle
8. t - test proportionally
9. Coefficient of asymmetry

Results

MRI analysis in patients with PTSD

Picture 1 Axial MRI scans - the slice on a hippocampal formation level

Picture 2 Axial MRI scans - length of hippocampal formation from the right and the left side
Table 1 Shows patients with PTSD towards simmetry/asimmetry hippocampal formation from right and left on the axial, coronal and sagittal slice

<table>
<thead>
<tr>
<th></th>
<th>Axial slice</th>
<th>Coronal slice</th>
<th>Sagittal slice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>in %</td>
<td>Number</td>
</tr>
<tr>
<td>SYMMETRY: hippocampal formation on the right and left side of the slice is the same size</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>ASYMMETRY hippocampal formation on the right and the left side of the slice is the different size</td>
<td>Total</td>
<td>10</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>Out of that:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The right side bigger than the left side</td>
<td>6</td>
<td>60.00</td>
<td>2</td>
</tr>
<tr>
<td>The left side bigger than the right side</td>
<td>4</td>
<td>40.00</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10</td>
<td>100.00</td>
<td>10</td>
</tr>
</tbody>
</table>

Length of hippocampal formation on the left and right side of axial slice are on all patient significantly asymmetric. Value of Chi - square test is: ChiSq = 10, level of assumen. is p < 0.01.
Length of hippocampal formation on the left and right side of sagittal slice is not statistically significant on the level of reliability p < 0.05. Value of ChiSq = 3.6. (Sign. for level p < 0.10).
At the coronary slice, there are no significant differences in patient proportion toward symm/asymm of width hippocampal formation with right and left side.

Table 2 Approximate size of the hippocampal formation on the right and left side on the axial, coronal and sagittal slice on patient with PTSD

<table>
<thead>
<tr>
<th>HIPPOCAMPAL FORMATION</th>
<th>Right side</th>
<th>Left side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X in cm</td>
<td>S.D. in cm</td>
</tr>
<tr>
<td>Axial slice</td>
<td>3.89</td>
<td>0.495</td>
</tr>
<tr>
<td>Coronal slice</td>
<td>2.08</td>
<td>0.116</td>
</tr>
<tr>
<td>Sagittal slice</td>
<td>4.08</td>
<td>0.477</td>
</tr>
</tbody>
</table>

Difference in average size of hippocampal formation between right and left side is not statistically significant for axial and coronal slice, but it is significant for sagittal slice.

Values of t- test are:

a) for axial slice: \( t = 0.0615 \) not significant
b) for coronal slice: \( t = 0.223 \) not significant
c) for sagittal slice: \( t = 2.727 \) significant p < 0.05
Picture 3
Sagittal MRI scans - the slice on a parahippocampal girus and hippocampal formation level

Picture 4
Sagittal MRI scans - size of amygdaloidal complex on the right and left side
**Table 3** Shows patients with PTSD towards symmetry/asymmetry amygdaloidal complex from right and left on the axial, coronal and sagittal slice

<table>
<thead>
<tr>
<th>SYMMETRY: Amygdaloidal complex on the right and left side of the slice is the same length</th>
<th>Axial slice (ant. — post. diameter)</th>
<th>Axial slice (lat.-med. diameter)</th>
<th>Sagittal slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>in%</td>
<td>Number</td>
<td>in%</td>
</tr>
<tr>
<td>2</td>
<td>20.00</td>
<td>2</td>
<td>20.00</td>
</tr>
</tbody>
</table>

**ASYMMETRY**
Amygdaloidal complex on the right and the left side of the slice is the different length

<table>
<thead>
<tr>
<th>Total:</th>
<th>Axial slice (ant. — post. diameter)</th>
<th>Axial slice (lat.-med. diameter)</th>
<th>Sagittal slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>in%</td>
<td>Number</td>
<td>in%</td>
</tr>
<tr>
<td>8</td>
<td>80.00</td>
<td>8</td>
<td>80.00</td>
</tr>
</tbody>
</table>

**Out of that:**

<table>
<thead>
<tr>
<th>The right side length than the left side</th>
<th>Axial slice (ant. — post. diameter)</th>
<th>Axial slice (lat.-med. diameter)</th>
<th>Sagittal slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>in%</td>
<td>Number</td>
<td>in%</td>
</tr>
<tr>
<td>5</td>
<td>50.00</td>
<td>6</td>
<td>60.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The left side length than the right side</th>
<th>Axial slice (ant. — post. diameter)</th>
<th>Axial slice (lat.-med. diameter)</th>
<th>Sagittal slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>in%</td>
<td>Number</td>
<td>in%</td>
</tr>
<tr>
<td>3</td>
<td>30.00</td>
<td>2</td>
<td>20.00</td>
</tr>
</tbody>
</table>

**TOTAL:**

<table>
<thead>
<tr>
<th>Number</th>
<th>in%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>100.00</td>
</tr>
</tbody>
</table>

On all three measurements, there are asymmetry in length of right and left side, and what is especially signed on sagittal slice.
Value Chi-squared test for sagittal slice is ChiSq = 10, level of reliability is p < 0.01.

**Table 4** Approximate length of the amygdaloidal complex on the right and left side on the axial and sagittal slice on patient with PTSD

<table>
<thead>
<tr>
<th>AMYGDALOIDAL COMPLEX</th>
<th>Right side (\bar{X}) in cm</th>
<th>S.D. in cm</th>
<th>Left side (\bar{X}) in cm</th>
<th>S.D. in cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial slice (ant.-post. diameter)</td>
<td>1.42</td>
<td>0.209</td>
<td>1.35</td>
<td>0.242</td>
</tr>
<tr>
<td>Axial slice (lat.-med. diameter)</td>
<td>1.88</td>
<td>0.275</td>
<td>1.82</td>
<td>0.200</td>
</tr>
<tr>
<td>Sagittal slice</td>
<td>1.17</td>
<td>0.205</td>
<td>1.15</td>
<td>0.191</td>
</tr>
</tbody>
</table>

Difference in average length of right and left side is not statistically significant for any case. Values of t-tests are:

a) For axial slice (ant. - post. diameter): \(t = 0.694\) not sign.

b) For axial slice (lat. - med. diameter): \(t = 0.549\) not sign

c) For sagittal slice: \(t = 0.226\) not sign.
Defining exact position of weak anatomic function which is find in a base of neurological and psychiatric disorder is just became the subject of intensive research interest. For this purposes it is important to implement structural and functional MRI techniques, also for further lightening and seeing subject of this work, more concretely connected to PTSD. Therefore, exactly MRI gives most sensitive volumetric measuring of hippocampus formation and amygdaloidal complex.

Karestan C. Koenen et al. - 2001. (20) Evaluate cognitive loss in PTSD. Authors mainly guess that many of PTSD symptoms are results of limbic structure hyperactivity, which can through their high-levelled projection in to prefrontal cortex contribute to dysfunction of this system and because of that, we can expect loss in performing of neuropsychological tasks. Result of newer researches that used MRI showed on smaller hippocampal size at PTSD (Van der Kolka 1996). Pitman, 1996, approved smaller hippocampal size bilaterally on to Vietnam war veterans with PTSD. Bremner et al., 1995, showed significant size decrease of right hippocampal volume on to Vietnam war veterans with PTSD. According to given results authors conclude that discontinuation in performing of neuropsychological tasks (O1 & IP), will show if limbic projection in to prefrontal cortex are going to be under attack with smaller hippocampal size in PTSD.

According to a basis of analyze of our results linked to size of hippocampal formation in all three projection (axial, coronary and sagittal), in a group of patient with PTSD, we can observe on a following way:

1. On to axial slice length of hippocampal formation on left and right side on all patients are significantly asymmetric. On to sagittal slice from the left side, the hippocampal formation is in many cases longer than right - 50 %.
   On coronary slice, there are no significant differences toward proportion patient according to symm. / asymm. of width of hippocampal formation on the left and right side.

2. Difference in average size of hippocampal formation between left and right side for axial and coronary slice is not statistically significant, but it is significant for sagittal slice.

According to analysis of our results connected to size of amygdaloidal complex in two projections (axial: ant. - post. in addition, lat. - med. diameter and sagittal), in a group of patient with PTSD, we can conclude that:

1. Patient with PTSD according to symm. / asymm. of amygdaloidal complex on the left and right side of axial and sagittal slice in all 3 measurement shows asymmetry, what is mostly present on sagittal slice.

2. Difference in average length of amygdaloidal complex on the left and right side is not statistically significant for none of the slices.

Conclusions

On basis of our analysis, we can conclude:

1. Every one of analyzed asymmetry shows the same characteristics in the group, in witch we emphasize variations.

2. We also emphasize the importance of presence of conciseness in individual characteristics of every one of the parameters in the shading light on asymmetry of hippocampal formation and amygdaloidal complex.

3. We have to be careful about what projection we are refusing to as being watched hippocampal formation and amygdaloidal complex because the results will depend on that. We can suggest the prospective studies in more projections because of the value of the statistically significant conclusions.

4. MRI - volumetric measurement have their value.

5. Usage of MRI techniques in examining the asymmetry of hippocampal formation and amygdaloidal complex, that we used, we suggest as the studies in the future research in the sense of the shading light on the anatomical functions that are based on neuropsychiatry dysfunctions.
References


Effects of the treatment of acute lumbar painful syndrome (ALPS) by "PRAXIS METHOD" during the period from 1996 to 2000

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1 Surgery "Praxis", Centre for Physical Medicine and Rehabilitation, Sarajevo
2 Faculty of Medicine, University of Sarajevo

Summary

Acute lumbar syndrome occurs suddenly and is accompanied with strong pain in the lower part of the back. The most frequent APLS causes are vertebral (herniation of intervertebral disc, subluxation of intervertebral disc, subluxation of intervertebral joint, fracture of vertebra - traumatic or pathological), or extravertebral (subluxation of sacroiliac joint, acute bursitis of iliolumbar segment, muscle injuries or injuries of tendo-ligamentous apparatus of lumbosacral region).

The treatment of acute lumbar painful syndrome is classified as medical, alternatively medical, surgical and combined. On the basis of durable experience, "Praxis method" as a treatment of lumbar pain (general and acute) is being applied in the Centre for Physical Medicine and Rehabilitation "Praxis" in Sarajevo. During the period from 1996 to 2000, the total number of 5,663 patients were examined in the centre "Praxis". Out of that number, 17.7% (1,003) of patients had acute lumbar painful syndrome (ALPS). Immediately after the therapeutic manipulation, which included "Praxis method, 31.5% (317) patients experienced the cessation of pains followed by ending of the treatment. The length of treatment for the rest of patients lasted: 1-7 days in 412 or 41.07% of patients, 8-21 days in 195 or 19.48% of patients, and more than 21 days in 79 or 7.88% of patients. For all patients (1,003) the average treatment duration was 6.6 days. The recidivation occurred in 127 patients (12.66%).

Throughout the treatment successfulness estimation according to clinical results scaled from 0 to 5, it was confirmed that out of the total number of 831 patients (82.85%) results were excellent in 459 patients (45.76%) or very good in 372 patients (35.09%).

The average age of patients was ranging between 35 and 45 years (621 patients or 61.9%). The male/female distribution was 2:1.

Key words: acute lumbar painful syndrome, "Praxis method".

Introduction

Acute lumbar syndrome problem solution (marked as M54.5 per X Revision of the international classification) is very delicate. Usually, the syndrome is accompanied with very strong pains and physical disablement. A acute lumbar painful syndrome is defined as a sudden, progressive pain in the lumbar region (sacral segment), with or without irradiation into lower extremities, and without neurological deficiency (1, 2, 3).

Lumbar painful syndrome represents the most frequent reason for patients' mostly work-active, visits to their physicians. Health care system, because of direct or indirect expenses, ensures the significant financial means for such patients (4, 5, 6).

According to the literature data (USA) lumbar pain makes 25% of all work injuries and causes the loss of 1400 working days on 1,000 employed persons a year (6, 7, 8, 9).

National statistics of European countries discovers that out of the 10 absences from work two relates to the persons with lumbar pain. The one-year prevalence is 25-45% while chronic lumbar pain occurs in 3-7% of adult persons (10, 11, 12).

Lumbar painful syndrome most frequently occurs in people of productive age (30-50 years of age). In the majority, it is accompanied with disablement to work and requires adequate medical treatment. The significant number of cases alters into the chronic form with prolongation of the painful phase, dysfunction, and working disablement (12).

Data on the frequency of lumbar painful syndrome in B&H are still being not particularly registered or systematically followed. Our own results of the treatment of patients with lumbar painful syndrome, during the period before 1992 and after 1995, show the identical lumbar painful syndrome trend as in other moderately developed countries (12).

Incidence of the patients with back pain is 5% a year. According to literature, 60-90% of adult persons experience back pain once in a life-time, while the pain tends to
repeat in 50% of these patients. According to Kenneth Mills, almost all persons older than 40 years have severe lumbar pains (4, 5, 13, 14).

Frequent lumbar painful syndrome occurrence in the world and in our country as well, required this study performance taking in consideration the cause of illness and the application of contemporary treatment. The reasons were not only medical, but also the economic ones.

Treatment of the lumbar painful syndrome according to various schools is applied by different methods and procedures. Operative procedure, performed when back pain is caused by hernia disc, is sufficiently frequent.

The long-term repeated stress, postural positional or sudden mechanical pressure, which does not immediately result in pains, can in the case of frequent subliminal microtrauma result in degeneration of the back and lumbar pain.

The special problem is a treatment of the acute lumbar syndrome, (marked as M54.5 according to X Revision of the International Classification) which is, because of its multi-causality, very delicate and often leads to physical disablement.

The most frequent APLS causes are vertebral (herniation of intervertebral disc, subluxation of intervertebral disc, subluxation of intervertebral joint, fracture of vertebral - traumatic or pathological), or extravertebral (subluxation of sacroiliac joint, acute bursitis of iliolumbar segment, muscle injuries or injuries of tendo-ligamentous apparatus of lumbosacral region).

Material and Methods

The sample for our research was differently aged persons treated because of the acute lumbar painful syndrome (APLS) in CBR "Praxis" during the period from January 1st, 1996 till December 31st, 2000. All of them suffered from APLS without radicular manifestation. APLS was treated by "Praxis" method based on the twenty-five years of experimental work of the author. Beside the classic treating methods, it included alternative medical procedures:

1. manual therapy,
2. local paravertebral application of the small dosages of depot corticoids,
3. rest in the phase of strong pains

Manual therapy, 1st to 3rd degree and strongly individually dosed, was applied in all cases of acute lumbar painful syndrome, with the resting up to 3 days and/or with the paravertebral application of depot corticoids.

After the painful phase, if the curing had not been achieved, a proper physical therapy was applied:

1. current analgesics for persisting pains,
2. manual massage,
3. dosed and specifically adjusted exercises (static and dynamic ones according to Brunk, Regan and Mecensi),
4. acupuncture in the case of persisting pain,
5. manual therapy (chiropractics) periodically as a recidivation prevention,
6. education for the performing of everyday exercises and for adoption of the protective positions by different activities.

All results are statistically processed and presented in tables and graphs.

The successfulness of treatment is expressed as an estimation of the clinical state of patients after the treatment according to scheme:

1. estimation "0": unchanged state (without treatment results),
2. estimation "2": minimal improvement,
3. estimation "3": satisfying functional improvement with sequelaes (sensor or motor ones),
4. estimation "4": well improvement and satisfying functional restitution with sequelaes,
5. estimation "5": good restitution without injury or disease consequences,
6. estimation "6": abandonment of treatment,
7. estimation "7": necessity for further medical treatment.

Patients treated in the centre "Praxis" were distributed into seven groups. Determined indexes were average age and mean data standard deviation. The same indexes were demonstrated throughout the estimation of result successfulness.

Results and discussion

In the observed five-years sample, out of 5663 patients with lumbar pain treated in the Centre for Physical Medicine and Rehabilitation "Praxis", the group M.54.5 of 1003 (17.71%) patients had acute lumbar pains (in accordance with the more recent literature).
Table 1 Summary review of all patients with lumbar painful syndrome treated in the centre "Praxis" during the period from 1996 to 2000

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>M51 Disc hernia</td>
<td>1431</td>
</tr>
<tr>
<td>M54.5 Acute LS</td>
<td>1003</td>
</tr>
<tr>
<td>M54.4 Chronic LS</td>
<td>1240</td>
</tr>
<tr>
<td>G55 Radicular syndrome</td>
<td>1025</td>
</tr>
<tr>
<td>G54.5 Lumboischialgic syndrome</td>
<td>964</td>
</tr>
<tr>
<td>TOTAL</td>
<td>5663</td>
</tr>
</tbody>
</table>

Graph 1 Summary review of all patients with lumbar painful syndrome treated in the centre "Praxis" during the period from 1996 to 2000

***

G54.5 Lumboischialgic syndrome 17%
M51 Disc hernia 25%
G55 Radicular 5y, 18%
M 54.4 Chronic LS 22%
M 54.5 Acute LS 18%

Graph 2 Sex structures of the patients with lumbar painful syndrome treated in the centre "Praxis" during the period from 1996 to 2000

***

Females 36%
Males 64%

Graph 3 Length of the treatment of patients treated in the centre "Praxis" during the period from 1996 to 2000

***

8-21 days 19%
> 21 days 8%
Instantly 32%
1-7 days 41%

The conclusion can be drawn according to two indexes:
1. number of treatment days, and
2. estimation of successfulness.

Table 2 Sex structures of the patients with lumbar painful syndrome treated in the centre "Praxis" during the period from 1996 to 2000

<table>
<thead>
<tr>
<th>M54.5</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute LS</td>
<td>638</td>
</tr>
<tr>
<td>Males</td>
<td>364</td>
</tr>
<tr>
<td>Females</td>
<td>364</td>
</tr>
<tr>
<td>Children up to 14 years of age</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1003</td>
</tr>
</tbody>
</table>

The 2:1 ratio for males (638 or 63.62%) and females (364 or 36.29%) with ALPS (Table 2 and Graph 2) indicates that male persons often work at more difficult physical jobs. This datum is in accordance with literature.

Table 3 Length of the treatment of patients treated in the centre "Praxis" during the period from 1996 to 2000; 6663 treatment days
Average = 6.6 days

<table>
<thead>
<tr>
<th>Instantly</th>
<th>1-7 days</th>
<th>8-21 days</th>
<th>&gt; 21 days</th>
<th>Totally</th>
<th>Recidivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>317</td>
<td>412</td>
<td>195</td>
<td>79</td>
<td>6663</td>
<td>12</td>
</tr>
<tr>
<td>Treatment days</td>
<td>317</td>
<td>1755</td>
<td>2237</td>
<td>2354</td>
<td>6663</td>
</tr>
</tbody>
</table>

Table 4 The results of the treatment of patients with acute lumbar syndrome treated in the surgery "Praxis" during the period from 1996 to 2000

<table>
<thead>
<tr>
<th>Per treatment results:</th>
<th>0</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>98</td>
<td>372</td>
<td>459</td>
<td>19</td>
<td>53</td>
</tr>
</tbody>
</table>
Graph 4  Review of the recidivation number in patients with acute lumbar syndrome treated in the surgery "Praxis" during the period from 1996 to 2000

Throughout the treatment successfulness estimation according to clinical results scaled from 0 to 5, it was confirmed that out the total number of 831 patients (82.85%) results were excellent in 459 patients (45.76%) or very good in 372 patients (35.09%). The majority of patients with ALPS, after the treatment in surgery "Praxis", had a good health restitution without sequelaes and recidivations. A good health improvement and satisfying functional restitution with minimal sequelaes were observed in the another group of patients what in overall makes more than four fifths of patients. A satisfying functional improvement was observed in 98 (9.78%) of patients having minimal sequelaes. The result was that 929 of patients (93.54%) successfully finished the treatment.

Beside the duration of treatment and the estimation of treatment successfulness, the age structure of patients is important from the economic point of view.

In our sample (Table 5 and Graph 6), the greatest number of patient were aged from 35 to 54 years (people of the most productive life age). The number of patients aged from 35 to 44 years was 352 or 35.09% and from 45 to 54 years the patients' number was 269 or 26.81%. The number of patient aged below 25 years and above 64 years abruptly decreased.

Table 5  Age structure of the patients with acute lumbar syndrome treated in the centre "Praxis" during the period from 1996 to 2000

<table>
<thead>
<tr>
<th>Per treatment results:</th>
<th>0</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>98</td>
<td>372</td>
<td>459</td>
<td>19</td>
<td>53</td>
</tr>
</tbody>
</table>

Conclusions

From the overall number of patients treated for the lumbar painful syndrome during the period of 5 years in the Centre for Physical Medicine and Rehabilitation 17.71% or 1003 had ALPS. Almost twice as many patients with ALPS were male patients. Exactly 317 (31.6%) of patients instantly recovered after the first treatment by therapeutic manipulation as a part of the "Praxis" method while 729 (72.68%) of patients recovered within the first 7 days.

Throughout the treatment successfulness estimation it was confirmed that 459 patients (45.76%) achieved good restitution without consequences, a good improvement with satisfying restitution and minimal sequelaes was observed in 372 patients and a satisfying functional improvement was observed in 929 (93.15%).

The majority of patients suffering from APLS belonged to the most productive life age.

Our results show, that the empiric method introduced as a doctrinaire one and applied in the treatment of patients with ALPS expressed a high degree of successfulness in the treatment of ALPS in comparison to the other methods. In the same time, such treatment had medical and economic advantages.
References

Diagnostic usefulness of serum carcinoembryonic antigen determinations in breast cancer patients

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Abstract

Background and purpose: Carcinoembryonic antigen (CEA) is used as a tumour marker in breast cancer (BC). In order to assess diagnostic value of CEA in BC we examined its serum levels and frequencies of its increase in breast cancer patients (BCP), and compared them to those in controls. We also determined CEA in patients with metastatic and non-metastatic BC, and calculated sensitivity and specificity of CEA in BC. Patients and methods: The main experimental group consisted of 47 female patients with histologically proved diagnosis of BC. There were two control groups: clinically healthy women, and female patients with other locations of cancer. Circulating levels of CEA were measured by means of immunoradiometric assay. Results were processed by means of t-test and two-way analysis of variance. Results: Circulating levels of CEA, before treatment in BCP, were significantly higher (p<0.0001) than in healthy women, and in patients with other cancers (p=0.007), while serum CEA in other cancer patients was significantly higher (p<0.01) than in healthy control. There was a difference between frequencies of CEA increase in BCP and healthy women, while such a difference did not exist between BCP and other cancer patients. The circulating levels of CEA in metastatic BCP were significantly higher (p=0.03) in comparison to non-metastatic patients. Sensitivity and specificity of CEA in BCP was 65.0%, and 57.1%, respectively. Conclusions: CEA does not have high tumour specificity for BC, since its circulating levels as well as frequencies of its increase may be elevated in patients with other types and locations of cancer, different from breast cancer. CEA can be detected in the serum of majority of patients with metastatic BC. CEA may be used as prognostic tumour marker in advanced BC.

Key words: carcinoembryonic antigen, sensitivity of CEA, specificity of CEA, localised breast cancer, metastatic breast cancer.

Introduction

Tumour markers are substances that can be detected in higher than normal amounts in the blood, urine, or body tissues of some people with certain types of cancer. A tumour marker may be produced by tumour itself or to a lesser extent by the body in response to cancer presence. Measuring their circulating levels may be very useful in clinical detection (diagnosis, screening), and management (monitoring, prognosis) of cancer patients. Carcinoembryonic antigen (CEA) is currently used among others as a tumour marker for breast cancer patients. It is a special protein that is actually produced by embryonic and regenerating cells, as well as, cancer cells. This protein belongs to the family of cell-surface

Table 1 The average circulating levels of CEA before treatment in healthy women (Control group I), patients with different locations and histologic types of cancer (Control group II) and women with breast cancer.

<table>
<thead>
<tr>
<th>Group / number of patients</th>
<th>Circulating levels of CEA (ng/mL) mean± s.e.</th>
<th>N patients / Total N patients (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group I 40</td>
<td>10.2 ± 0.8 a</td>
<td>40/40 (100)</td>
<td>&lt; 0.0001 (c versus a)</td>
</tr>
<tr>
<td>Control group II 33</td>
<td>28.1 ± 4.4 b</td>
<td>16/33 (48.5)</td>
<td>&lt; 0.007 (c versus b)</td>
</tr>
<tr>
<td>Breast cancer patients 47</td>
<td>69.4 ± 13.8 c</td>
<td>18/47 (38.3)</td>
<td>&lt; 0.01 (b versus a)</td>
</tr>
</tbody>
</table>

< upper referent value (21 ng/mL) N patients / Total N patients (%)>

> upper referent value (21 ng/mL) N patients / Total N patients (%)
glycoproteins and can be measured in blood, as cells shed these proteins. CEA is a circulating antigen expressed by human breast cancer cells but it is also commonly associated with colorectal cancer. Non-cancerous conditions such as stomach ulcers, colon polyps, cigarette smoking, may also cause elevations of this protein.

In order to assess diagnostic value of CEA determination in breast cancer patients we examined CEA circulating levels and frequencies of its increase in breast cancer patients and compared them to those in healthy women and in patients with cancers of different histologic origin and location. Furthermore, we determined CEA serum levels in patients with metastatic breast cancer and compared them to those in patients with localized breast cancer. We also calculated diagnostic sensitivity and specificity of CEA for both breast cancer patients and other cancer patients.

Patients and methods

Patients

There were two control groups of patients designated as I, and II. Group I consisted of forty clinically healthy women with an age interval 30-65 years from whom the following data have been collected: name, age, place of birth, place of residence, and life habits. All of them were non-smokers, non-obese, and were not under any medication including birth control. All of them were occasional coffee drinkers. All of them had normal mammograms. The circulating levels of CEA were measured in all of them at least two times during the observation period.

Group II consisted of 33 female patients having cancer of different histologic origin and location with an age interval 18-77 years. Majority of them (45.7%) had lung cancer, then gastrointestinal (24.4%), urinary bladder (6.7%), skin (6.7%), uterine (6.7%), laryngeal (3.3%), and bone cancer (3.3%), and cancer with unknown primary location (6.7%). The same data were collected as in group I. The circulating levels of CEA at the time of initial diagnosis before any treatment (signed as baseline) were measured in all of them, and later again at least two times during the observation period. According to the presence of metastases this group was further divided into two subgroups: patients without metastases, and those with metastases.

The main experimental group consisted of 47 female histologically confirmed breast cancer patients with an age interval 38-82 years. The same type of data has been taken from them as in group II. This group was further divided according to presence of metastases into two subgroups: patients with metastases, and those without metastases. The same group was also divided according to their serum CEA measured before the onset of treatment: hyperCEA with average circulating levels of CEA of more than 21 ng/mL, and normo and hypo CEA having average circulating levels less than 21 ng/mL.

Methods

Determination of serum levels of CEA

Blood samples were drawn under sterile conditions at eight in the morning each time, centrifuged at 3000 rpm for ten minutes under room temperature and serum was stored in plastic tubes at -200C until processed. The circulating levels of CEA were determined by means of immunoradiometric assay using commercially available kits from Biomedica (Graz, Austria). The major characteristics of this method are: principle - immunoradiometric assay; separation method - coated beans; antibodies - monoclonal on solid phase; labeler - 125-I-labeled monoclonal antibodies; incubation - 2 hours on 370 C; standards - 0, 5, 10, 35 and 70 ng/mL; sensitivity - 0.25 ng/mL; specificity - no cross reactions for hemolysed or lipemic samples; normal values 2-21 ng/mL.

Estimation of range of normal values for CEA

We first estimated the range of normal values in 40 healthy female subjects. Our referral values were mean + 2 standard deviations. The values of normal ranges stretched between 2.5-20.7 ng/mL. There were no age dependent significant differences in CEA concentrations. All cases with their values of CEA above 21 ng/mL were judged as hyperCEA, and all of them with their values of CEA below 2.5 ng/mL were declared as hypoCEA.

Determination of sensitivity and specificity of CEA

All individual values of CEA concentrations were judged based on and according above mentioned lower and upper values. The sensitivity and specificity were calculated according to the following formulas:

Sensitivity = true positive results / (true positive + false negative results)
Specificity = true negative results / (true negative + false positive results)

Statistical analysis of results

The results were evaluated using student's t-test with calculated standard errors and two standard deviations, and analysis of two-way variance in F-test. The nature of distribution and linearity were checked using histogram presentation, and tests of linearity including Kolmogorov-Smirnov Z (with kurtosis and skews), with probability and de-trended probability plots. These methods were done using the Statistical Package for Social Sciences (SPSS) program. The statistical significance of
Results

The circulating levels of CEA

Table 1 shows the average circulating levels of CEA and frequency of their increase before treatment in breast cancer patients compared to two control groups (healthy women and other cancer patients).

Circulating levels of CEA before treatment in breast cancer patients were highly significantly elevated ($p<0.0001$) than in healthy women, and very significantly elevated ($p<0.007$) than in patients with other locations and histologic types of cancer before treatment. Serum CEA levels before treatment in other cancer patients were significantly higher ($p<0.01$) than in healthy women. None of women from healthy control group was hyperCEA, but 17 (51.5%) from 33 other cancer patients, and 29 (61.7%) from 47 breast cancer patients had hyperCEA levels. There was a difference between frequencies of CEA increase in breast cancer patients and in healthy women while such a difference did not exist between breast cancer and other cancer patients.

The circulating levels of CEA in localised and in metastatic breast cancer

Metastases were not detected in seven (14.8%), and were detected in forty (85.2%) of breast cancer patients during the five-year follow up period (table 2). Elevated levels of CEA were detected in the majority (72.5%) of breast cancer patients with metastases. The average circulating levels of CEA in metastatic breast cancer patients were significantly higher ($p<0.03$) in comparison with non-metastatic patients (table 2), while in patients with other types and locations of cancer such a difference did not show up. The sensitivity / specificity for CEA attest to these results.

Table 2 The average serum levels of CEA before treatment in patients with localised and advanced breast cancer.

<table>
<thead>
<tr>
<th>Group / number of patients</th>
<th>Serum level of CEA (ng/mL) mean +/- S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer patients without metastases 7</td>
<td>24.9 +/- 12.5 $a$</td>
</tr>
<tr>
<td>Breast cancer patients with metastases 40</td>
<td>66.4 +/- 2.8 $b$</td>
</tr>
<tr>
<td>Other cancer patients without metastases 10</td>
<td>21.9 +/- 8.7 $c$</td>
</tr>
<tr>
<td>Other cancer patients with metastases 23</td>
<td>33.3 +/- 8.8 $d$</td>
</tr>
</tbody>
</table>

$a$ versus $b$ $p<0.03$
$c$ versus $d$ $p<0.36$

Specificity and sensitivity for CEA determination in breast cancer patients

Table 3 shows the results of calculated sensitivity/specificity for CEA in breast cancer patients. Sensitivity for CEA in metastatic breast cancer was 65.0%, and specificity was 57.1%.

Specificity and sensitivity for CEA determination in other cancer patients

Table 4 shows the results of calculated sensitivity and specificity for CEA in patients with other types and locations of cancer. Sensitivity for CEA in this group of cancer patients was 60.9%, and specificity was 70.0%.

Table 3 The sensitivity and specificity of CEA determinations in breast cancer patients.

<table>
<thead>
<tr>
<th>Group of patients</th>
<th>Number of patients with hyperCEA</th>
<th>Number of patients with normo and hypoCEA</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with metastases</td>
<td>TP (26)</td>
<td>FN (14)</td>
<td>TP/TP+FNx100</td>
<td>TN/TN+FPx100</td>
</tr>
<tr>
<td>Number of patients without metastases</td>
<td>FP (3)</td>
<td>TN (4)</td>
<td>26/40x100 = 65</td>
<td>4/7x100 = 57.1</td>
</tr>
</tbody>
</table>

TP = True positives, number of patients with metastases correctly classified by the test.
FP = False positives, number of patients without metastases miss-classified by the test.
FN = False negatives, number of patients with metastases miss-classified by the test.
TN = True negatives, number of patients without metastases correctly classified by the test.
Discussion

In spite the fact that there was a significant difference (p<0.007) between the baseline circulating levels of CEA before treatment in breast cancer patients and in patients having cancers of other types and locations, there was not a significant difference when the frequencies of its elevated levels were compared between these two groups of patients. Furthermore majority of patients with cancers of other types and locations (51.5%) had elevated CEA in their blood (table 1 and 4). These findings can be explained by the fact that the most of patients with other cancers had colorectal, gastric or lung cancer. It is well known that CEA is commonly associated with these types of cancer, and, therefore, its high levels does not necessarily indicate that a woman has breast cancer. These data may indicate that CEA doesn't have high tumour specificity for breast cancer, which makes its diagnostic usefulness less valuable.

Diagnostic usefulness of tumour marker CEA was also assessed on the basis of sensitivity and specificity of its determination in breast cancer patients. Sensitivity or true-positive rate was in fact the frequency of CEA elevated levels in breast cancer patients with metastases, and it was 65% (table 3), while specificity or true-negative rate was the frequency of normal CEA levels in non-metastatic breast cancer patients (e.g. possibility for false positive results in all unexpected cases of high circulating levels of CEA) and it was 57.1% (Table 3). Sensitivity and specificity of CEA in control group of patients with other types and locations of cancer were not significantly different in relation to breast cancer patients. These results together with those before about the baseline CEA levels in breast cancer patients indicate that CEA doesn't have high tumour specificity for breast cancer. This in fact means that blood levels of CEA may be elevated in patients with other types and primary locations of malignant tumours different from breast cancer. Our findings about sensitivity of CEA for metastatic breast cancer are in accordance with other results published before (1, 2, 3, 4, 5, 6, 7).

The circulating levels of CEA in breast cancer patients with metastases were significantly higher than in those without metastases (table 2), while such a difference did not exist in the control group of patients with other types and locations of cancer. This difference points towards the diagnostic importance of CEA in detection of advanced breast cancer, and in monitoring the results of treatment. Breast cancer patients especially those with metastases had significantly higher serum CEA levels as compared to the controls and those with localised disease, irrespective to the site of metastases (8). The other studies have also shown that circulating CEA tend to be elevated in women with advanced breast cancer (9,10, 11). CEA can be detected in serum of majority of patients with metastatic breast cancer. Since increasing serum levels were shown to be associated with clinical manifestation and progression of metastases, CEA may be used as a marker in metastatic breast cancer. Measurements of circulating CEA may be adequate and useful tool for the follow up and early diagnosis of metastases in breast cancer patients (9, 12). These findings also support the opinion that CEA is a tumour antigen of less differentiated cells. The cells of the metastatic cancer are usually poorly differentiated and size of both primary and metastatic tumour is mainly dependent on the proliferation fraction inside the tumour. Probably the number of metastatic deposits consisting of de-differentiated cells plays here a major role.

Table 3 The sensitivity and specificity of CEA determinations in breast cancer patients.

<table>
<thead>
<tr>
<th>Group of patients</th>
<th>Number of patients with hyperCEA</th>
<th>Number of patients with normo and hypoCEA</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with metastases</td>
<td>TP (14)</td>
<td>FN (9)</td>
<td>TP/TP+FNx100</td>
<td>TN/TN+FPx100</td>
</tr>
<tr>
<td>Number of patients without metastases</td>
<td>FP (3)</td>
<td>TN (7)</td>
<td>14/23x100= 60.9</td>
<td>7/10x100= 70.0</td>
</tr>
</tbody>
</table>

TP, FP, FN, and TN are explained in the legend of Table 3.
References


Abstract

Research on the parameters of full blood count and differential white blood count is included in the program of all medical laboratories of primary, secondary and tertiary health care levels. Today, all haematological tests are exclusively performed on the haematology analyzers. Automation of haematology laboratories is a result of the huge requirements for haematological test performing, timely issuing of the haematological findings, and possibility of the usage of modern techniques.

This work is an evaluation of laser haematology analyzer Cell-Dyn 3700 SL. It investigates the reliability of test results throughout the following parameters: precision, accuracy, sensitivity and specificity of determination methods. It also explores the influence of sample transferring and correlation with haematology analyzer MAXM Retti. Haematology parameters that have been investigated are: white blood cell (WBC), neutrophils (NEU), lymphocytes (LXM), monocytes (MONO), eosinophils (EOS), basophils (BASO), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCHC) red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), plateletocrit (PCT), and platelet distribution width (PDW).

The results confirm that precision of analyzer fulfils the reproducibility of testing parameters: WBC, RBC, HGB, MCV, MCH, MCHC, and PLT. Correlation coefficient values (r) gained throughout the statistical analysis, that is linear regression results obtained throughout the comparison of two analyzers are adequate except for MCHC (r = 0.64), what is in accordance with literature data.

Accuracy is tested by haematology analyzer method and microscopic differentiating method. Correlation coefficient results for granulocytes, lymphocytes and monocytes point the accuracy of methods. Sensitivity and specificity parameters fulfil the analytical criteria. It is confirmed that haematology analyzer Cell-Dyn 3700 SL is reliable for the determination of full blood count in everyday work. A analyzer and its program for differential white blood count can be used for the research and separation of normal and pathological blood counts with addition of microscopic methods confirming distribution or morphologic changes of leukocytes.

Introduction

Laboratory investigation of the count, concentration and relative relation of haematological parameters is extremely important in clinical and other researches. Determination of the full blood count and differential white blood count is included in the program of all medical laboratories of primary, secondary, and tertiary health care levels (1).

Manual techniques have been performed in haematological laboratories for a long time. These techniques are slow and strenuous while their subjectively based test estimation and work precision like white blood cells count on haemocytometer are not on the acceptable level. Today, haematological tests are being performed on haematology analyzers. Started in 1960's, automation in haematology laboratories has been developed very fast during the last 20 years. The reasons for that are increasing demands established in haematological laboratories; timely issuing of the large number of haematological findings and attaining a high-level of the accuracy and precision in laboratory work.

Microprocessors and computers, as integral part of haematological counter, induced a sudden development of automation and an expansion of different types of haematological tests (2). New generation of haematology analyzers enable fast and reliable obtaining of the full blood count data and differential white blood count information screening. Data on abnormalities in distribution, that is changes in relations between normal leukocyte types helps in diagnosing and following up of different disorders such as haematological and infectious disease processes.

The basic goal of determination of the differential white blood count on haematology analyzer is a decrement in usage of manual microscopic differentiation in routine work. The usage of instruments provides a proper estimation of whether there is a normal or susceptible pathological differential white blood count, which needs to be accurately examined later on.

Cell-Dyn 3700 SL is haematology analyzer manufactured by Abbott-USA. It measures 17 blood parameters including differential white blood count. This work investigates the precision, accuracy, sample transferring, sensitivity, and specificity of haematology analyzer Cell-Dyn 3700 SL and evaluates its usage and reliability in everyday work.
Material and methods

Instrument

Haematology analyzer Cell-Dyn 3700 SL measures 17 blood parameters at the same time: white blood cell (WBC), number and percentage of neutrophils (NEU), number and percentage of lymphocytes (LYM), number and percentage of monocytes (MONO), number and percentage of eosinophils (EOS), number and percentage of basophils (BASO), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCHC) red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), plateletocrit (PCT), platelet distribution width (PDW).

Analyzer works according to two principles - volumetric impedance and optical detection. There are 4 independent measurements: leukocyte counting and differentiation performed by optical principle (diffraction light usage), leukocyte counting performed by impedance principle in the current canal (measurement of single cell electrical resistance), erythrocyte and platelet counting performed in the independent canal and haemoglobin measurement performed by spectrophotometer. White blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), and platelets (PLT) are measured directly, but other parameters (HTC, MCV, MCH, MCHC, RDW, and PDW) are calculated automatically from the measured parameter data. Haemoglobin is measured by spectrophotometer according to cyanmethemoglobin method at wavelength of 540 nm.

Principle of the five-parts differential white blood count is based on the erythrocyte membrane lysis done by haemolysing reagents and followed by the differentiation of leukocyte types (NEU, LYM, MONO, EOS and BASO) according to size of the cell or nucleus. These leukocyte types are detected by peaks and size of histograms and are distributed into 5 groups.

For the full blood cell count analysis on Cell-Dyn 3700 SL on Open Mode procedure it's necessary to collect 130 L of the full blood while 240 L of the full blood for Close Mode procedure. Apparatus receives previously mixed blood and during the routine analyzing procedure is possible to analyze an urgent sample, as well. These operations are completely automatic. Ten stands with ten test tubes of blood each - 100 samples in each series can be placed in the porter (mixer) of samples. Instrument can store up to 10 000 sample (patient) data. Besides numerical data, analyzer provides histograms of the leukocyte, erythrocyte and platelet distribution. Its informative system has a quality control program that provides monitoring process of the apparatus accuracy and reproducibility in everyday work.

Chemicals

The original regent sets and control blood from manufacturer Abbott are used:

- Diluent Cell-Dyn Reagent, 99231,
- Sheath Reagent, 99311,
- Detergent Cell-Dyn Diff Screen Reagent, 99321,
- Lyse Reagent, 99431,
- Control Blood, Tri Level Control, 22PA.

Blood sample

Venous blood of the patients from Clinics of Clinical Centre of University of Sarajevo, (N= 219) have been sampled by vacutainer blood tubes (Becton Dickenson Vacutainer Systems) of 3.0 mL containing K3EDTA as an anticoagulant. All samples have been collected from ambulance patients and patients with different diseases hospitalised in Clinical Centre. Samples have been stored at room temperature.

Precision

The precision inside series has been tested by consecutive measurements (N=25) of the same blood sample according to haematological parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT.

The precision between series for the same parameters has been tested by analysing of the series of samples (N =20) during the different time periods. First series of samples has been tested after the blood collection and 2 hours later, second series has been tested after the blood collection and 5 hours later, and third series has been tested after the blood collection and 10 hours later.

Sample transferring

Sample transferring has been performed according to method introduced by Broughton and associates.
sample with high level of analytes has been consequent-
ly measured for three times (a1, a2, a3) and after that a
sample with low level of analytes has been consequently
measured for three times (b1, b2, b3). Percentage of the
transferring of each sample analyte has been calculated
by formula:
\[
\frac{b_1 \times b_1}{a_3 \times b_3} \times 100
\]

Accuracy

The accuracy of measurements has been tested by paral-
lel measuring of 65 full blood samples according to
haematological parameters: WBC, RBC, HGB, HCT,
MCV, MCH, MCHC and PLT on haematology analyzers
Cell-Dyn 3700 SL Abbott and MAXM Retti Coulter.
Both analyzers have been previously calibrated by the
same universal calibrator and checked by the same con-
trol.
Parallel measuring of 93 blood samples on haematology
analyzer Cell-Dyn 3700 SL A bbott and manual micro-
scopic leukocyte differentiating has been performed in
order to investigate the accuracy of measurements of the
differential white blood count parameters. Microscopic
differentiating of blood smear coloured by Pappenheim
method has been performed on 100 cells (6).

Sensitivity and specificity

Sensitivity and specificity of haematology analyzer Cell-
Dyn 3700 SL have been tested by comparison of the dif-
ferential white blood count results (N=93) gained on ana-
lyzer and throughout microscopic differentiating.

Statistics

Results have been statistically evaluated and expressed
by means of standard deviation (SD), mean value (X),
and coefficient of variation (CV). Congruency of results
has been investigated by analysis of the linear regression
and expressed as a coefficient of correlation (r).

Results and discussion

Precision testing

M easurement of the the same sample (N=25) according
to haematological parameters: WBC, RBC, HGB, HCT,
MCV, MCH, MCHC and PLT has been performed for the
precision testing. Mean value (X), standard deviation
(SD), and coefficient of variation (CV) have been calcu-
lated for every parameter. Statistical parameters are
shown in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\bar{X}$</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC ($10^9$/L)</td>
<td>8.17</td>
<td>0.18</td>
<td>2.21</td>
</tr>
<tr>
<td>RBC ($10^{12}$/L)</td>
<td>5.47</td>
<td>0.05</td>
<td>1.04</td>
</tr>
<tr>
<td>HGB (g/L)</td>
<td>162</td>
<td>1.84</td>
<td>1.22</td>
</tr>
<tr>
<td>HCT (L/L)</td>
<td>0.46</td>
<td>0.01</td>
<td>1.05</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>83.9</td>
<td>0.53</td>
<td>0.73</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>29.6</td>
<td>0.22</td>
<td>0.78</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>353</td>
<td>3.10</td>
<td>0.92</td>
</tr>
<tr>
<td>PLT ($10^9$/L)</td>
<td>26.6</td>
<td>9.70</td>
<td>3.62</td>
</tr>
</tbody>
</table>

Coefficient of variation data ranging from 0.73 for MCV
to 3.62 for PLT points the acceptable reproducibility for
every tested parameter. They are within the span recom-
mended by Cell-Dyn 3700 SL manufacturer. Figure 2
presents a graphic survey of the coefficient of variation
values for the tested parameters.

Figure 2  Graphic survey of the coefficient of vari-
ation values for the tested parameters

Precision testing results between three series (N=20) for
parameters WBC, RBC, HGB, HCT, MCV, MCH,
MCHC and PLT are expressed as a coefficient of corre-
lation (r) and shown in Table 2. Graphic survey of these
parameters is presented in Figure 3.

All coefficient of correlation-results for tested parameters
(in the function of time - 0h and 2h, 0h and 5h and 0h and
10h) are acceptable except for MCV (r = 0.77), probably
as a consequence of erythrocyte swelling after the sample
10-hours storage at room temperature.
Table 2  Precision testing results - coefficient of correlation (r) between series

<table>
<thead>
<tr>
<th>Time</th>
<th>WBC</th>
<th>RBC</th>
<th>HGB</th>
<th>HCT</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
<th>PLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0^a and 2^a</td>
<td>0.99</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
<td>0.97</td>
<td>0.96</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>0^a and 5^a</td>
<td>0.99</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.95</td>
<td>0.95</td>
<td>0.96</td>
<td>0.98</td>
</tr>
<tr>
<td>0^a and 10^a</td>
<td>0.99</td>
<td>0.95</td>
<td>0.97</td>
<td>0.88</td>
<td>0.77</td>
<td>0.92</td>
<td>0.82</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Figure 3  Graphic survey of precision - coefficient of correlation (r) between series

![Graphic survey of precision - coefficient of correlation (r) between series](image)

Sample transferring testing

The results of the sample transferring influences during the continuous measurement of haematological parameters WBC, RBC, HGB, PLT, GR and LYM are shown in Table 3. Percentage data on sample transferring are below 1% what means that sample transferring is insignificant for all tested parameters.

Accuracy testing

For accuracy testing of the blood count parameters, 65 full blood samples have been simultaneously tested on Cell-Dyn 3700 SL and MAXM Retti analyzers. Correlation coefficient values (r) obtained throughout statistical analysis and linear regression comparative results (between two analyzers) are shown in Figure 4.

Correlation coefficient values for the tested parameters are satisfactory except for MCHC (r = 0.64), what is in accordance with literature data (7). Low correlation coefficient values for MCHC (up to r = 0.15(3)) could be found in numerous scientific publications. Authors have utilized different haematology counters and analyzers. Some authors explained MCHC low results as a consequence of the non-existence of two identical analyzers with equal measurement fissures resulting with the different values of indirect, calculated parameters. It is important to mention that correct calculation of the MCHC value requires values of erythrocyte number, haemoglobin concentration, and haematocrit result.

For accuracy testing of the differential white blood count parameters, 93 full blood samples have been tested on Cell-Dyn 3700 SL analyzer and by microscopic differentiating method. In this case, we have statistically processed three leukocyte series: absolute number of granulocytes (NEU + EOS + BASO), lymphocytes and monocytes (because we have considered that 93 blood samples are not sufficient for the statistical calculation of five-part differential white blood count (NEU, LYM, MONO, EOS and BASO). Linear regression two-method analysis for three, above-mentioned parameters is shown in Figure 5.

Linear regression data have acceptable correlation coefficient values for granulocytes (r = 0.977), lymphocytes (r = 0.973) and monocytes (r = 0.976), what is in accordance with literature data (8).

Table 3  The sensitivity and specificity of CEA determinations in breast cancer patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High level</th>
<th>Low level</th>
<th>Transferring (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x 10^9/L)</td>
<td>13.7</td>
<td>3.05</td>
<td>0.91</td>
</tr>
<tr>
<td>RBC (x 10^{12}/L)</td>
<td>6.54</td>
<td>1.80</td>
<td>0.64</td>
</tr>
<tr>
<td>HGB (g/L)</td>
<td>195</td>
<td>34</td>
<td>0.85</td>
</tr>
<tr>
<td>PLT (x 10^9/L)</td>
<td>806</td>
<td>59</td>
<td>0.55</td>
</tr>
<tr>
<td>GR (%)</td>
<td>83</td>
<td>10</td>
<td>0.41</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>57</td>
<td>6</td>
<td>0.26</td>
</tr>
</tbody>
</table>
Figure 4 linear regression comparative results between two analyzers Cell Dyn 3700 SL and MAXM Retti according to parameters WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT.
Sensitivity and specificity

Sensitivity and specificity of haematology analyzer Cell-Dyn 3700 SL according to the differential white blood count parameters have been performed by differentiation analysis on apparatus and microscopically. Same blood samples used for accuracy testing have been also used for the testing of reliability parameters. Truly negative result (TN) and truly positive result (TP) have been calculated according to relation for sensitivity $\frac{TP}{(TP+FN)} \times 100$ and relation for specificity $\frac{TN}{(TN+FP)} \times 100$. Sensitivity and specificity testing results are shown in Table 4.

Table 4 Testing parameters for the sensitivity and specificity of method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cell Dyn 3700 SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>62</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
</tr>
</tbody>
</table>

Truly negative result (TN) means a non-pathological differential white blood count result having a good differentiation correlation on analyzer and microscopically. We have obtained 62 (66.6%) truly negative results throughout comparison of the differential white blood counts. Truly positive result (TP) means a pathological differential white blood count result having a good differentiation correlation on analyzer and microscopically. We have obtained 28 (30.1%) truly positive results meaning the presence of immature cells (myelocyte, metamyelocyte and lymphoblast).

False negative result (FN) means a differential white blood count result not marked as a pathological on analyzer while microscopic sample result was different. Only 1 sample (1.07%) out of 93 tested samples had a false negative result.

False positive result (FP) means a differential white blood count result marked as a pathological on analyzer while microscopic sample result was different. Two samples (2.15%) out of 93 tested samples had false positive results.

In comparison with literature data, our correlation for the differential white blood count are better. Especially good is correlation between manual differentiation and analyzer differentiation with samples having no morphologic or distributive abnormalities of leukocytes.

Results of this evaluation indicate that this type of samples can be differentiated with confidence on analyzer in Figure 5 Analysis of the linear regression comparative results of differential white blood count on Cell-Dyn 3700 SL and by manual differentiating method.
everyday work. On the contrary, when analyzer indicates any kind of abnormalities in blood cell composition or analyzer warning system is on, a sample has to be microscopically tested. When it is already known or the analyzer "warns" on the pathological morphology of blood cells, a differential white blood count result obtained on the analyzer needs to be processed microscopically, particularly in the cases of acute infectious processes, haematological diseases and allergy accompanied with haematology parameter changes. In all cases, only microscopic differentiation provides the correct differential white blood count findings. In addition, all cases of the impossibility of proper recognition of the leukocyte morphological abnormalities (the presence of immature leukocytopoiesis cells, lithoplasma and granulation changes, and the presence of toxic granulations) demand microscopic blood sample differentiation. During the process of differentiation of differential white blood count on analyzer 7 warning cases (7.5%) have been found and later processed microscopically.

**Conclusion**

Automatic laser haematology analyzer Cell-Dyn 3700 SL is a reliable haematology analyzer for the determination of full blood count in everyday work. Analyzer and its program for differential white blood count could be used for the research and separation of normal from pathological blood counts. For that reason, it is possible to decrease significantly everyday manual differentiation, particularly differentiation of the blood samples from healthy persons who have no morphological or distributive abnormalities in leukocytes. Together with the interpretation principle of results obtained on analyzer and respecting of analyzer "warning" system, it is still necessary to perform the microscopic method of leukocyte differentiating, particularly in detection of new haematological diseases and control of the previously detected and treated cases.

References


Classification and evaluation of medical devices

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Abstract

Medical devices and medical disposables contribute significantly to the quality and effectiveness of the health care system. It is necessary to commit scientifically sound regulatory environment that will provide consumers with the best medical care. This includes continued services to small manufacturers, readily available guidance on FDA requirements, predictable and reasonable response times on applications for marketing, and equitable enforcement. But in the public interest, this commitment to the industry must be coupled with a reciprocal commitment: that medical device firms will meet high standards in the design, manufacture, and evaluation of their products. The protections afforded our consumer, and the benefits provided the medical device industry, cannot be underestimated.

Key words: medical devices, classification, manufacture, evaluation

Introduction

Medical devices are an extraordinarily heterogeneous category of products. The term "medical device" includes technologically simple articles such as hypodermic syringes and blood bags. On the other end of the spectrum are highly sophisticated articles such as pacemakers, surgical lasers, implantable pumps, and vascular grafts.

Medical devices and medical disposables contribute significantly to the quality and effectiveness of the health care system. Medical devices range from wound dressings to artificial hearts, designed to support life in many end-stage cardiac patients. The Food and Drug Administration (FDA) estimates that some 2700 medical devices and over 1500 medical disposables are used yearly. Biomaterials represent the fundamental reason for this impressive performance. In the early 1930s the only "biomaterials" were wood, glass, and metals. These were used mostly in surgical instruments, paracorporeal devices, and disposable products. The advent of synthetic polymers changed the entire character of health care delivery. Polymers originally designed for commercial applications were adapted for implantable prostheses, thus opening the way for pacemakers, vascular grafts, synthetic wound dressings that mimic intact human skin, and a variety of artificial organs (1).

Classification

FDA has established classifications for approximately 1,700 different generic types of devices and grouped them into 16 medical specialties. Each of these generic types of devices is assigned to one of three regulatory classes based on the level of control necessary to assure the safety and effectiveness of the device. The three classes and the requirements which apply to them are:

Class I devices (general controls) are intended primarily for applications that pose no potential risk to health, and thus can be adequately regulated without imposing standards or the need for premarket review. This category provides a broad general control. It requires that manufacturers register these devices with the FDA, provide a listing of products, maintain adequate reports, and comply with Good Manufacturing Practices (GMPs). Examples include stethoscopes, periodontic syringes, nebulizers, vaginal insufflators.

Class II devices (performance standards) are applicable when general controls are not adequate to assure the safety and effectiveness of a device, based on the potential risk to health posed by it. To classify a device in the Class II category, the FDA must find that enough data are available on which to base adequate performance standards that would control the safety and effectiveness of these devices. Examples include diagnostic catheters, electrocardiographs, wound dressings, percutaneous catheters, gastrointestinal irrigation systems.

Class III devices (premarket approval) include "critical devices," that is, life-supporting and life-sustaining devices, unless adequate justification is given for classification in another category. After 1976, Class III contained devices that are not sufficiently similar to pre-1976 devices, and devices that were regulated as new drugs before 1976. Examples include bronchial tubes, ventilators, vascular grafts, pacemakers, cardiopulmonary bypass, surgical meshes, and others.

In the past, medical devices, for the most part, were simple instruments such as stethoscopes and scalpels in which defects would be readily apparent. The technology boom after World War II, greatly increased the number and complexity of medical devices, including landmark products such as heart-lung machines and dialysis equipment.
According to the technical definition, a "device" is "an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part or accessory, which is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or intended to affect the structure or any function of the body and which does not achieve its primary intended purposes through chemical action and which is not dependent upon being metabolized for the achievement of its primary intended purposes."

As this definition suggests, many different types of products are properly regulated as medical devices. Medical devices include over 100,000 products in more than 1,700 categories. These range from simple everyday articles such as thermometers, tongue depressors, and heating pads, to the more complex devices such as pacemakers, intrauterine devices, fetal stents and kidney dialysis machines.

Although some of the earliest medical devices (e.g. bandages) have retained their same basic form and function, the complexity and use of medical devices have increased exponentially over the past sixty years. Patient care has improved dramatically as a result of these changes. The following examples illustrate advances that have been made in medical technology in just the last few years:

- heart defibrillators have progressed from large, bulky external pumps to small external machines to totally implantable devices;
- surgical tools enable to operate on a fetus in utero;
- coronary artery disease that once required open heart surgery has been largely replaced by less invasive techniques such as balloon angioplasty, insertion of cardiovascular stents, laser ablation of plaque and minimally invasive surgery;
- devices that are more sophisticated, more dependable and more convenient;
- "artificial" skin for burn victims is now available.
- many major surgical procedures (e.g., removal of the gallbladder) have been replaced with laparoscopic procedures that require only small incisions. This "revolution" alone has dramatically reduced hospital stays and recuperation is much faster.
- new devices have been developed to do needle biopsy of breast abnormalities without general anesthesia or major surgery;
- many diagnostic devices now can be used at home, e.g., testing for blood clotting, pregnancy, cholesterol, glucose, genetic diseases;
- improvements to anesthesia systems have reduced risks to patients several-fold.
- new imaging systems (PET and MRI) provide a dramatic improvement in image quality, information, content and analysis.
- cemented joint replacements for hips have given way to better functioning, more durable replacements, not just for hip problems but for nearly every joint in the body.

FDA has also approved several breakthrough devices:

- the Thoratec ventricular assist device system, for example, is a pump that assists the heart in patients who are waiting for a heart transplant and are at imminent risk of dying before a donor heart is available;
- the Ultramark high definition ultrasound system is a first-of-a-kind device to aid the physician in differentiating benign from malignant breast lesions;
- the PAPNET testing system is an aid in re-screening Pap smears previously reported as negative.

As diverse as medical devices are, so are the range and complexity of problems that can arise from their use.

These problems include:

- mechanical failure,
- faulty design,
- poor manufacturing quality,
- adverse effects of materials implanted in the body,
- improper maintenance/specifications,
- user error,
- compromised sterility/shelf life and
- electromagnetic interference among devices.

Examples of injuries resulting from use of medical devices include:

- bone disintegration caused by the material used in temporomandibular jaw implants;
- patient deaths caused by fractures in implanted artificial heart valves; and
- electrocution of babies when apnea monitor leads were mistakenly plugged into wall outlets.

The 1938 Act initially charged FDA with removing adulterated or misbranded medical devices from the market. It did not give the Agency the authority to review medical devices before entering the market. Changes were made in the Act in 1976 after a commission determined that more than 700 deaths and 10,000 injuries were associated with medical devices. After concluding that the Act did not provide sufficient authority for the FDA adequately to protect the public health with respect to medical devices, the Medical Device Amendments of 1976 were passed (1976 Amendments) (2).
The 1976 Amendments provided several mechanisms to achieve this goal, including classification of medical devices, device listing, establishment registration, adherence to Good Manufacturing Practices (GMPs), and extensive control over market introduction of medical devices. The Safe Medical Devices Act of 1990 (3) and the Medical Device Amendments of 1992 (4) revised and expanded the 1976 Act.

The Agency carries out its medical device responsibilities by:

- evaluating new products before they are marketed for conformance to requisite design, engineering bench tests, and, as needed, data from animal trials or clinical trials in patients;
- assuring quality systems are in place in the device manufacturing plants—through inspection and enforcement activities; and,
- collecting and monitoring adverse effects from marketed products and investigations, and taking action, when necessary, to prevent injury or death;

The process provides for orderly development of new devices starting with bench and animal tests, moving next through scientifically sound clinical investigations, and, only after independent review of the results, approval for marketing.

This system has three goals:

(1) to screen out bad ideas and products that are unsafe or don't produce a benefit;
(2) to provide early feedback in order to detect and fix design or manufacturing flaws; and
(3) to give doctors and patients an accurate interpretable experience from which to determine in whom to use a device, what to expect from its use, and how to avoid a prolonged learning curve using it (so that patients benefit).

Evaluating new devices before they are marketed

Because of the diverse nature of devices and the device industry, it is necessary to have a product approval system with special characteristics. In the USA there is a classification system of products based on the degree of risk and the need for information on use of the device in patients.

Devices on the market at the time the original law was passed were assigned to one of three "classes." Those presenting the least risk, such as elastic bandages, were placed in Class I and subject to "general controls." General controls include registration and listing, prohibitions against adulteration and misbranding, notification, repair/replace/ refund, recall, records and reports, and adherence to Good Manufacturing Practices (GMPs). Although a number of Class I devices still require premarket notification, approximately three-fourths are low risk devices that FDA has exempted from premarket notification. Examples of such devices include oxygen masks and manual surgical instruments such as scalpels and tissue retractors.

Class II devices, presenting greater concern, are subject to "special controls" such as postmarket surveillance studies and performance standards, in addition to the general controls. On the risk spectrum these are the next category of devices about which the technology is well understood but we need to review data about the performance of the device, usually through bench test data. The highest risk devices are those that represent new technology. These are Class III devices, which include many implanted and life-supporting or life-sustaining devices, are subject to more stringent controls and requirements, including premarket review. For these devices, comprehensive evaluation, including data from clinical studies, is required to ensure safety and effectiveness. This involves bench and animal tests, clinical trials, the submission of a Premarket Approval Application (PMA), and in many cases review by an outside advisory panel. Examples of devices in this category include heart valves, implantable defibrillators, and computerized microscopes that automatically read Pap smears.

New devices are classified automatically into Class III and require approval unless they are either shown to be substantially equivalent to another device for which premarket approval is not required or they are reclassified. The vast majority of devices (approximately 98%) enter the market through this premarket notification process. Examples include hearing aids; hip implants; CT, ultrasound, x-ray, and MRI imaging devices; and surgical lasers.

Quality systems for device manufacture

FDA inspects manufacturing facilities to be sure they are in compliance with "good manufacturing practices" (GMPs). FDA published a quality system regulation (21 CFR Parts 808, 812 and 820) (5) which revised GMPs by adding design control requirements. The new quality systems regulation will enhance consumer protection by reducing the number of recalls from poorly designed devices and resultant patient injuries. It has been estimated that nearly half of the 1200 device product recalls conducted annually are attributed to device design. The new regulations also are consistent with quality system requirements worldwide; this meets an important goal of global harmonization.
Adverse effects reporting

Postmarket surveillance of already-marketed devices is a vital complement to the premarket review program, because no system of premarket review, no matter how thorough, can prevent all potential safety problems once a device is in widespread use. The regulation of medical devices presents unique challenges. To address these challenges requires both breadth and depth of scientific capabilities. The FDA must maintain staffing and expertise of the following scientists in order to keep pace with advances:

- Engineers (including biomedical, electrical/electronics, and materials).
- Biologists and microbiologists
- Physicians and other clinicians
- Chemists, biochemists, and toxicologists
- Pharmaceutical technologists
- Physicists
- Statisticians
- Consumer safety officers and field investigators
- Human factors specialists

Conclusion

It is necessary to commit scientifically sound regulatory environment that will provide consumers with the best medical care. This includes continued services to small manufacturers, readily available guidance on FDA requirements, predictable and reasonable response times on applications for marketing, and equitable enforcement. But in the public interest, this commitment to the industry must be coupled with a reciprocal commitment: that medical device firms will meet high standards in the design, manufacture, and evaluation of their products. The protections afforded our consumer, and the benefits provided the medical device industry, cannot be underestimated.

References

3. The Safe Medical Devices Act of 1990 (Public Law 101-629)
4. The Medical Device Amendments of 1992 (Public Law 102-300)
5. Quality system regulation (21 CFR Parts 808, 812 and 820)
At this moment, public health authorities, physicians and scientists around the world are struggling to cope with a severe and rapidly spreading new disease in humans called severe acute respiratory syndrome, or SARS. According to World Health Organisation (WHO) this appears to be the first severe and easily transmissible new disease to emerge in the 21st century. Though much about the disease remains poorly understood, including the details of the causative virus, we do know that it has features that allow it to spread rapidly along international air travel routes.

As of 10 May 2003, a cumulative 7296 probable SARS cases with 526 deaths have been reported from 30 countries on three continents (WHO, ProMED). In the past week, more than 1000 new probable cases and 96 deaths were reported globally. This represents an increase of 119 new cases and 8 new deaths compared with 9 May 2003 (China (85), Taiwan (23), and Hong Kong (7) represented the overwhelming majority, with one additional case each reported from France, Malaysia, Singapore, and the United States). Only in China, as of 10 May 2003 (WHO) total of 4884 with 235 deaths have been reported. Some outbreaks have reassuring features.

SARS historical overview

Severe Acute Pulmonary Syndrome (SARS) was first identified in Viet Nam on 28 Feb 2003, when Dr Carlo Urbani, an epidemiologist from the Hanoi WHO office, examined a patient with a severe form of pneumonia for which no aetiology could be found. On 10 Mar 2003, 22 hospital workers in Hanoi French Hospital were ill with a similar acute respiratory syndrome, and by 11 Mar 2003, similar outbreaks were reported among hospital workers in Hong Kong.

SARS occurred at a time of heightened surveillance for atypical respiratory disease that affected health workers, their families and contacts in Guangdong Province, with (at that time) 305 cases and 5 deaths reported from 16 Nov 2002 to 7 Feb 2003. Approximately 30 percent of cases were reported to occur in health care workers. Surveillance was heightened further when a 33-year-old man who had travelled with his family to Fujian Province in China died in Hong Kong on 17 Feb 2003. The next day, Hong Kong authorities announced that avian influenza A (H5N1) virus, the cause of "bird flu", had been isolated from both the man and his 9-year-old hospitalized son. Another member of the family, an 8-year-old daughter, died in Fujian and was buried there.

On 12 Mar 2003, after an assessment of the situation in Asia with WHO teams in Hanoi, Hong Kong, and Beijing, a global alert was issued about cases of severe atypical pneumonia with unknown aetiology that appeared to place health workers at high risk.

Two days later, on 14 Mar 2003, WHO received a report from the government of Canada that health authorities had taken steps to alert hospital workers, ambulance services, and public health units across the provinces that there were four cases of atypical pneumonia within a single family in Toronto that had resulted in two deaths.

At 0200h Geneva time 15 Mar 2003, the government of Singapore notified WHO, by urgent telecommunication, of a similar illness in a 32-year-old physician who had treated hospital workers with a severe respiratory syndrome in Singapore, including one from the French Hanoi hospital who had self-evacuated to Singapore. This Singapore physician had travelled to the United States for a medical conference, and at the end of the conference boarded a return flight to Singapore in New York. Before departure, he had indicated to a colleague in Singapore by telephone that he had symptoms similar to the patients he had treated in Singapore. The colleague notified health authorities and WHO identified the airline and flight, and the physician and his accompanying family members were removed from the flight at a stopover.
in Frankfurt, Germany, where he was immediately isolated and placed under hospital care, as were his 2 accompanying family members when they developed fever and respiratory symptoms several days later. As a result of this prompt action, Germany experienced no further spread linked to the three imported cases.

Later in the morning of 15 Mar 2003, with this background and chronology of events, a decision was made by WHO to increase the level of the global alert issued on 12 Mar 2003. The decision was based on 5 different but related factors:

- First, the aetiology, and therefore the potential for continued spread, of this new disease were not yet known.
- Second, the outbreaks appeared to pose a great risk to health workers who managed patients, and to the family members and other close contacts of patients.
- Third, many different antibiotics and antiviral agents had been tried empirically and did not seem to have an effect.
- Fourth, though the numbers were initially small, a significant percentage of patients (25 of 26 hospital staff in Hanoi, and 24 of 39 hospital staff in Hong Kong) had rapidly progressed to respiratory failure, requiring intensive care and causing some deaths in previously healthy persons.
- Finally, the disease had moved out of its initial focus in Asia and appeared to have spread to North America and Europe.

At this time, the epidemiology of SARS was poorly understood. A virulent strain of influenza had not been ruled out as a possible cause, even though transmission patterns were not characteristic for influenza. There was also some hope that the new disease, like many other new diseases of the recent past, would fail to maintain efficient person-to-person transmission, or that it might attenuate with passage and eventually self-contain. Despite the lack of understanding about the disease, its cause, and future evolution, the need was great to introduce a series of emergency measures to contain SARS outbreaks in the affected areas and prevent further international spread, thus reducing opportunities for the new disease to establish endemology. WHO thus decided, on 15 Mar 2003, to issue a rare emergency travel advisory as a global alert to international travellers, health care professionals, and health authorities?

At the same time, the global alert recommended no change in patterns of international travel, but that passengers notify their health authority if they should develop signs and symptoms and have a history of travel to areas reporting cases of SARS. Following this alert, awareness increased immediately, and many potential new outbreaks were prevented by the prompt isolation and strict management of suspected cases.

By 27 Mar 2003, however, it was evident that international spread of SARS had continued after the 15 Mar 2003 advisory at 2 of the earliest outbreak sites, namely Viet Nam and Hong Kong. Persons on the same airplanes as persons with symptoms consistent with SARS, and sitting in close proximity to them, had developed signs and symptoms compatible with SARS. On this date, it was decided to recommend new measures related to international travel, still with the intent of preventing the international spread of the infectious agent. These recommendations were that SARS-affected areas, where chains of human-to-human transmission were known to occur, institute measures to identify international passengers who had signs, symptoms and history compatible with SARS, and to recommend that such persons postpone international travel and seek medical advice. These recommendations were instituted in most of the affected areas shortly after 27 Mar 2003.

Concern however, continued to mount. An urgent investigation of the Amoy Gardens, outbreak in Hong Kong began on 29 Mar 2003, and the following day, health officials announced that 213 Amoy residents were probable cases of SARS. This followed an unusual cluster of cases, closely linked in time and place, among guests and visitors who had stayed on the same floor of a hotel located in the same district (Kowloon) as Amoy Gardens. By this same date, nine business travellers and tourists had returned to Singapore, Beijing, and Taiwan from Hong Kong, either sick or in the incubation period of SARS.

Outbreaks in the hotel and housing estate indicated that SARS was showing an unusual pattern of transmission in Hong Kong, probably involving an environmental component that would place persons at risk outside the confined health care settings associated with outbreaks in most other countries. The nine cases of probable SARS that occurred in Singapore, Beijing, and Taiwan associated with travel in Hong Kong, indicated that the risk of international spread was continuing.

Cases of possible transmission in airplanes continue to be reported and investigated. As recently as 5 Apr 2003, notification of a SARS patient travelling internationally by sea from Hong Kong to Vladivostok (Russian Federation) was received, opening a possible second route of international travel for the virus.

WHO travel recommendations are kept under constant review and will be amended as more data about the evolution of SARS become available.
Aetiology of SARS

On 17 Mar 2003, a network of 11 leading laboratories around the world was set up as a mechanism for expediting identification of the SARS causative agent. Today, after joining of two more laboratories from China during first part of April, there are total 13 laboratories from 10 countries all around the World who are working on SARS virus research. Laboratories were selected based on three criteria:

- outstanding scientific expertise,
- facilities at biosafety level III, and
- capacity to contribute to the battery of tests and experiments that would be needed to fulfil Koch’s four postulates for the identification of an infectious agent as the cause of a specific disease.

The network was set up on the model of the influenza network and provides another important lesson: models and systems set up for one health emergency can be rapidly adapted to serve others.

Collaboration is virtual. Members of the network confer in daily teleconferences coordinated by WHO and use a secure web site to post electron microscopic pictures of candidate viruses, sequences of genetic material for virus identification and characterization, descriptions of experiments, and results. The well-guarded secret techniques that give each laboratory its competitive edge have been immediately and openly shared with others. Laboratories also quickly exchange various samples from patients and post-mortem tissues. These arrangements have allowed the analysis of samples from the same patient simultaneously in several laboratories specialized in different approaches, with the results shared in real time. This collaboration has resulted in the identification of the suspected causative agent, and the development of three diagnostic tests, with unprecedented speed.

Recent findings in China show that with seven investigated fatal cases both Chlamydia like and Coronavirus like agents were found in all seven cases of atypical pneumonia collected in Guangdong province. Since the Chlamydia-like agents presenting in both organs and cell cultures could not react with the genus specific antibodies against Chlamydia and monoclonal antibodies against C. pneumoniae and C. psittaci, the results might well be suggestive of a novel Chlamydia-like agent (Hong T. et al, 2003), and not only a new Coronavirus. Virus isolation continues from patients with SARS, and at the same time virus has been isolated from tears and faeces. Publications on these various findings are being prepared by members of this collaborating group, but the need remains for a highly sensitive and specific PCR test to diagnose acute infections.

New coronavirus discovered

Through new mechanisms set up by WHO, progress in research has been unprecedented, particularly in the rapid discovery of a new coronavirus and development of diagnostic tests. The best scientists from around the world are working on these problems around the clock, and in an unprecedented spirit of collaboration against a threat of as-yet-unknown dimensions. Nonetheless, we still do not have conclusive proof that the new virus is indeed the cause of SARS. The results of animal experiments, which are currently being conducted by a laboratory in a WHO network, will be available soon and may provide the last pieces of evidence needed for definitive proof that SARS is caused by the newly discovered coronavirus. The family of Coronaviridae consists of enveloped, generally spherical virions with helical nucleocapsids containing a single, positive stranded RNA genome. Coronavirus are shown to have a high mutation rate, thus rapidly producing new, genetically differing variants or subspecies. This special behaviour may complicate the development of valuable diagnostic test systems showing sufficient sensitivity and specificity. Furthermore, the findings will provide additional evidence to understand the role of metapneumovirus as a possible “helper virus” in persons co-infected with the new coronavirus.

Diagnostic tests

The development of a diagnostic test has proved more problematic than hoped. Three tests are now available and are helping to improve understanding of how the virus causes disease in humans. However, all three tests have limitations as tools for bringing the SARS outbreak under control.

The ELISA detects antibodies reliably but only from about day 20 after the onset of clinical symptoms. It therefore can not be used to detect cases at an early stage before they have a chance to spread the infection to others. The second test, an immunofluorescence assay (IFA), detects antibodies reliably but only from day 10 of infection, but is a comparatively slow test that requires the growth of virus in cell culture.

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1 These postulates stipulate that to be the causal agent, a pathogen must meet four conditions:
1. it must be found in all cases of the disease,
2. it must be isolated from the host and grown in pure culture,
3. it must reproduce the original disease when introduced into a susceptible host, and
4. it must be found in the experimental host so infected.
The current RT-PCR molecular test for detection of SARS virus genetic material is useful in the early stages of infection but produces many false negatives. This means that many persons who actually carry the virus may not be detected - creating a dangerous sense of false security in relation to a virus that is known to spread easily in close person-to-person contact.

Present role of tests in diagnosis
A positive test result indicates that a person is (RT-PCR), or recently was (ELISA, IFA), infected with the coronavirus. However, a negative test result does not guarantee that the person is not infected with the virus.

At present, reporting to WHO of probable SARS cases is based on an assessment of clinical symptoms, history -- including travel history -- of possible exposure to an infected person, and distinctive chest X-rays. The 10 countries in the WHO laboratory network, namely Canada, France, Germany, Japan, Hong Kong SAR, the Netherlands, Singapore, the United Kingdom, and the United States of America, are beginning to conduct routine laboratory testing of suspect and probable SARS cases. WHO has posted on its web site details about the test methodology that allows other countries to perform tests. However, more work is needed to produce a robust test that is capable of rapidly and reliably detecting cases at an early stage of infection.

Epidemiology of SARS

Modes of transmission
A collaborative group on epidemiology, made up of investigators from all sites with local transmission of SARS, continues to confirm person-to-person transmission as the major route of transmission. Based on the latest findings of virus survival in the environment (See Table 1.) there probably is more than one way of SARS transmission.

The WHO team also found evidence of "super-spreaders" in Guangdong, including one who is thought to have infected as many as 100 other persons. The outbreak dates back to 16 November 2002, when an initial case was reported in Foshan City. The phenomenon of a "super-spreader", which is not a recognized medical condition, also dates back to the early days of the outbreak. At that time, when SARS was just being recognized as a severe new disease, many patients were thought to be suffering from atypical pneumonia from other causes, and were therefore not treated as special cases requiring special precautions of isolation and infection control. In SARS outbreaks, a "super-spreader" is a source case who, for yet unknown reasons, has infected a large number of persons. It remains unknown whether such "super-spreaders" are persons secreting an exceptionally high amount of infectious material or whether some other factor, perhaps in the environment, is working to amplify transmission at some key phase of virus shedding (WHO).

Incubation period
SARS appears to be less infectious than influenza. The incubation period is short, estimated to range from 2-7 days, with maximum of 10 days (WHO), with 3-5 days being more common. However, the speed of international travel creates a risk that cases can rapidly spread around the world. One recently published analysis of data from Hong Kong estimates a longer maximum incubation period in a group of 57 patients. The longer incubation period could reflect differences in methodology, specificity of diagnosis, route of transmission, infectious dose, or other factors. Reliable diagnosis - determining that all cases diagnosed as SARS are true cases of the disease - has been particularly difficult to establish in this outbreak, as diagnosis is based on a set of non-specific symptoms and clinical signs that are seen in several other diseases.

Case fatality ratio
On 7 May 2003 WHO revised its initial estimates of the case fatality ratio of SARS. The revision is based on an analysis of the latest data from Canada, China, Hong Kong SAR, Singapore, and Viet Nam. On the basis of more detailed and complete data, and more reliable methods, WHO estimates that the case fatality ratio of SARS ranges from 0% to 50% depending on the age group affected, with an overall estimate of case fatality of 14% to 15%. Based on data received by WHO until 7 May, the case fatality ratio is estimated to be less than 1% in persons aged 24 years or younger, 6% in persons aged 25 to 44 years, 15% in persons aged 45 to 64 years, and greater than 50% in persons aged 65 years and older. One method of overcoming this difficulty is to calculate the case fatality ratio using only those cases whose final outcomes - died or recovered - is known. However, this method, when applied before an outbreak is over, gives an overestimate because the average time from illness

2 A case fatality ratio measures the proportion of all people with a disease who will die from the disease. In other words, it measures the likelihood that a disease will kill its host, and is thus an important indicator of the severity of a disease and its significance as a public health problem. The likelihood that a person will die of SARS could be influenced by factors related to the SARS virus, the route of exposure and dose (amount) of virus, personal factors such as age or the presence of another disease, and access to prompt medical care. Many factors compound efforts to calculate a case fatality ratio while an outbreak is still evolving. Deaths from SARS typically occur after several weeks of illness. Full recovery may take even longer. While an epidemic is still evolving, only some of the individuals affected by the disease will have died or recovered. Only at the end of an epidemic can an absolute value be calculated, taking into account total deaths, total recoveries and people lost to follow-up. Calculating case fatality as the number of deaths reported divided by the number of cases reported irrespective of the time elapsed since they became ill gives an underestimate of the true case fatality ratio.

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onset to death for SARS is shorter than the average time from illness onset to recovery. With these methods, estimates of the case fatality ratio range from 11% to 17% in Hong Kong, from 13% to 15% in Singapore, from 15% to 19% in Canada, and from 5% to 13% in China. A more accurate and unbiased estimation of case fatality for SARS can be obtained with a third method, survival analysis. This method relies on detailed individual data on the time from illness onset to death or full recovery, or time since illness onset for current cases. Using this method, WHO estimates that the case fatality ratio is 14% in Singapore and 15% in Hong Kong. In Viet Nam, where SARS has been contained and measurement is more straightforward, case fatality was comparatively low, at 8%. One explanation for this is the large number of total cases that occurred in younger, previously healthy health care workers.

Prevention

A high awareness of SARS symptoms among travellers and the medical and nursing professions has often resulted in good management of imported cases - prompt isolation of patients and management according to strict procedures of infection control. WHO continues to recommend the earliest possible isolation of all suspect and probable cases of SARS. A short time between onset of symptoms and isolation reduces opportunities for transmission to others. It also reduces the number of contacts requiring active follow-up, and thus helps relieve some of the burden on health services. In addition, prompt hospitalization gives patients the best chance of receiving possibly life-saving care should their condition take a critical course. As a result, many countries having only a single or a few imported cases have experienced no further spread to hospital staff, families of patients and hospital visitors, or the community at large. SARS patients should be placed in an isolation unit. Strict respiratory and mucosal barrier nursing is recommended. It is very important that suspected cases are separated from other patients and placed in their own hospital room. Health care workers and visitors should wear efficient filter masks, goggles, aprons, head covers, and gloves when in close contact with the patient. (WHO: Hospital Infection Control Guidance).

On 1 May 2003, WHO updated SARS case definition (WHO). The surveillance case definitions, based on available clinical and epidemiological data, are now being supplemented by a number of laboratory tests and will continue to be reviewed as tests currently used in research settings. They will become more widely available as diagnostic tests. Preliminary clinical description of Severe Acute Respiratory Syndrome summarizes what is currently known about the clinical features of SARS. Countries may need to adapt case definitions depending on their own disease situation. Retrospective surveillance is not expected. Clinicians are advised that patients should not have their case definition category downgraded while awaiting results of laboratory testing or on the bases of negative results (WHO: Use of laboratory methods for SARS diagnosis).

Suspect case

1. A person presenting after 1 November 2002\(^3\) with history of:
   - high fever (>38 °C)
   AND
   - cough or breathing difficulty
   AND one or more of the following exposures during the 10 days prior to onset of symptoms:
   - close contact\(^4\) with a person who is a suspect or probable case of SARS;
   - history of travel to an area with recent local transmission of SARS
   - residing in an area with recent local transmission of SARS

2. A person with an unexplained acute respiratory illness resulting in death after 1 November 2002, but on whom no autopsy has been performed

IN ADDITION, one or more of the following exposures during to 10 days prior to onset of symptoms:
   - close contact\(^4\), with a person who is a suspect or probable case of SARS;
   - history of travel to an area with recent local transmission of SARS
   - residing in an area with recent local transmission of SARS

Probable case

1. A suspect case with radiographic evidence of infiltrates consistent with pneumonia or respiratory distress syndrome (RDS) on chest X-ray (CXR).
2. A suspect case of SARS that is positive for SARS coronavirus by one or more assays. (WHO Use of laboratory methods for SARS diagnosis).
3. A suspect case with autopsy findings consistent with the pathology of RDS without an identifiable cause.

\(^3\) The surveillance period begins on 1 November 2002 to capture cases of atypical pneumonia in China now recognized as SARS. International transmission of SARS was first reported in March 2003 for cases with onset in February 2003.

\(^4\) Close contact: having cared for, lived with, or had direct contact with respiratory secretions or body fluids of a suspect or probable case of SARS.
Exclusion criteria

A case should be excluded if an alternative diagnosis can fully explain their illness.

Reclassification of cases

As SARS is currently a diagnosis of exclusion, the status of a reported case may change over time. A patient should always be managed as clinically appropriate, regardless of their case status.

- A case initially classified as suspect or probable, for which an alternative diagnosis can fully explain the illness, should be discarded after carefully considering the possibility of co-infection.
- A suspect case that, after investigation, fulfils the probable case definition should be reclassified as "probable".
- A suspect case with a normal CXR should be treated, as deemed appropriate, and monitored for 7 days. Those cases in which recovery is inadequate should be re-evaluated by CXR.
- Those suspect cases in whom recovery is adequate but whose illness cannot be fully explained by an alternative diagnosis should remain as "suspect".
- A suspect case who dies, on whom no autopsy is conducted, should remain classified as "suspect". However, if this case is identified as being part of a chain transmission of SARS, the case should be reclassified as "probable".
- If an autopsy is conducted and no pathological evidence of RDS is found, the case should be "discarded".

Reporting procedures

- All probable SARS cases should be managed in the same way for the purposes of infection control and outbreak containment (WHO: Management of Severe Acute Respiratory Syndrome (SARS)).
- At this time, WHO is maintaining surveillance for clinically apparent cases only i.e. probable and suspect cases of SARS. (Testing of clinically well contacts of probable or suspect SARS cases and community based serological surveys are being conducted as part of epidemiological studies which may ultimately change our understanding of SARS transmission. However, persons who test SARS CoV positive in these studies will not be notified as SARS cases to WHO at this time).
- Where laboratory tests are not available or not done, probable SARS cases as currently defined above should continue to be reported in the agreed format.
- Suspect cases with positive laboratory results will be reclassified as probable cases for notification purposes only if the testing laboratories use appropriate quality control procedures.
- No distinction will be made between probable cases with or without a positive laboratory result and suspect cases with a positive result for the purposes of global surveillance. WHO will negotiate sentinel surveillance of SARS with selected partners to collect detailed epidemiological, laboratory and clinical data.
- Cases that meet the surveillance case definition for SARS should not be discarded on the basis of negative laboratory tests at this time.

Rationale for retaining the current surveillance case definitions for SARS

The reason for retaining the clinical and epidemiological basis for the case definitions is that at present there is no validated, widely and consistently available test for infection with the SARS coronavirus. Antibody tests may not become positive for three or more weeks after the onset of symptoms. It is currently undetermined if all patients will mount an antibody response. Molecular assays may not be positive in the early stages of illness using currently available reagents. No one is yet able to define the optimal specimen to be tested at any given stage of the illness. This information is accruing as more tests are being performed on patients with known exposures and/or accompanied by good clinical and epidemiological information. It is hope that in the near future an accessible and validated diagnostic assay(s) will become available which can be employed with confidence at a defined, early stage of the illness.

First data on stability and resistance of SARS coronavirus compiled by members of WHO laboratory network (WHO)

Virus survival in stool and urine

- Virus is stable in faeces (and urine) at room temperature for at least 1-2 days.
- Virus is more stable (up to 4 days) in stool from diarrhoea patients (which has higher pH than normal stool).

Disinfectants and fixatives (for use in laboratories)

- Virus loses infectivity after exposure to different commonly used disinfectants and fixatives.

Virus survival in cell-culture supernatant

- Only minimal reduction in virus concentration after 21 days at 4°C and -80°C.
- Reduction in virus concentration by one log only at stable room temperature for 2 days. This would
indicate that the virus is more stable than the known human coronaviruses under these conditions.  
- Heat at 56°C kills the SARS coronavirus at around 10000 units per 15 min (quick reduction).

Full research results from four different hospitals are presented in Table 1.

**SARS: a particularly serious threat to international health**

Although the last decades of the previous century witnessed the emergence of several new diseases, SARS needs to be regarded as a particularly serious threat for several reasons. If the SARS virus maintains its present persistence, it could become a global health crisis.

**Table 1.** Research results from four different hospitals on virus survival time in an environment

<table>
<thead>
<tr>
<th>Lab*</th>
<th>Substrate</th>
<th>Initial viral count log_{OPFU}</th>
<th>Condition</th>
<th>Survival time</th>
<th>Method of testing viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Government Virus Unit, Department of Health, Hong Kong, SAR China</td>
<td>virus spiked in baby stool</td>
<td>1.00E+03</td>
<td>pH 6-7</td>
<td>3 hr</td>
<td>Virus isolation in cell culture</td>
</tr>
<tr>
<td></td>
<td>virus spiked in normal stool</td>
<td>7.50E+03</td>
<td>pH 8</td>
<td>6hr</td>
<td>Virus isolation in cell culture</td>
</tr>
<tr>
<td></td>
<td>virus in diarrhoeal stool</td>
<td>7.50E+03</td>
<td>pH 9</td>
<td>4days</td>
<td>Virus isolation in cell culture</td>
</tr>
<tr>
<td></td>
<td>Stool</td>
<td>1.00E+03</td>
<td>Room Temperature</td>
<td>at least 2 days</td>
<td>Virus isolation in cell culture</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>1.00E+03</td>
<td>Room Temperature</td>
<td>at least 24 hr</td>
<td>Virus isolation in cell culture</td>
</tr>
<tr>
<td></td>
<td>Virus culture medium+ 1% bovine serum</td>
<td>1.00E+03</td>
<td>on plastic surface in room temperature</td>
<td>at least 2 days</td>
<td>Virus isolation in cell culture</td>
</tr>
<tr>
<td>Queen Mary Hospital, The University of Hong Kong, Hong Kong, SAR China</td>
<td>Virus culture medium+ 1% bovine serum</td>
<td>1.00E+04</td>
<td>30-37°C</td>
<td>at least 1hr</td>
<td>Virus isolation in cell culture</td>
</tr>
<tr>
<td></td>
<td>Virus culture medium+ 1% foetal calf serum</td>
<td>1.00E+04</td>
<td>56°C</td>
<td>degradation of titre over time (10 000 infectious virus units in 15 min)</td>
<td>Virus isolation in cell culture</td>
</tr>
<tr>
<td></td>
<td>virus in Acetone, 10% Formaldehyde and Parafomaldehyde, 10% Clorox, 75% ethanol, 2% phenol</td>
<td>1.00E+06</td>
<td>Room Temperature</td>
<td>less than 5 min</td>
<td>Virus isolation in cell culture</td>
</tr>
<tr>
<td>National Institute of Infectious Diseases, Tokyo, Japan</td>
<td>Virus culture+ 2% bovine serum</td>
<td>1.00E+06</td>
<td>minus 80°C</td>
<td>at least 4 days</td>
<td>Virus isolation and RT-PCR</td>
</tr>
<tr>
<td></td>
<td>Virus culture+ 2% foetal calf serum</td>
<td>1.00E+06</td>
<td>4°C</td>
<td>at least 4 days</td>
<td>Virus isolation and RT-PCR</td>
</tr>
<tr>
<td></td>
<td>Virus culture+ 2% foetal calf serum</td>
<td>1.00E+06</td>
<td>37°C</td>
<td>less than 4 days</td>
<td>Virus isolation and RT-PCR</td>
</tr>
<tr>
<td></td>
<td>Virus culture+ 2% foetal calf serum</td>
<td>1.00E+05</td>
<td>56°C</td>
<td>less than 30min</td>
<td></td>
</tr>
<tr>
<td>University Marburg, Germany</td>
<td>Virus culture</td>
<td>1.00E+06</td>
<td>4°C</td>
<td>at least 21 days</td>
<td>Virus isolation</td>
</tr>
<tr>
<td></td>
<td>Virus culture</td>
<td>1.00E+06</td>
<td>minus 80°C</td>
<td>at least 21 days</td>
<td>Virus isolation</td>
</tr>
</tbody>
</table>

**Source:** WHO
pathogenicity and transmissibility, SARS could become the first severe new disease of the 21st century with global epidemic potential. As such, its clinical and epidemiological features, though poorly understood, give cause for particular alarm. With the notable exception of AIDS, most new diseases that emerged during the last 2 decades of the previous century or established endemology in new geographical areas have features that limit their capacity to pose a major threat to international public health. Many (Avian influenza, Nipah virus, Hendra virus, Hanta virus) failed to establish efficient human-to-human transmission. Others (Escherichia coli O157:H7, variant Creutzfeldt-Jakob disease) depend on food as a vehicle of transmission. Diseases such as West Nile Fever and Rift Valley Fever that have spread to new geographical areas require a vector as part of the transmission cycle and are associated with low mortality, often in high-risk groups, such as the elderly, the immunocompromised, or persons with co-morbidity. Still others (Neisseria meningitidis W135, and the Ebola, Marburg, and Crimean-Congo haemorrhagic fevers) have strong geographical foci. Although outbreaks of Ebola haemorrhagic fever have been associated with case-fatality rates in the range of 53 percent (Uganda) to 88 percent (Democratic Republic of the Congo), person-to-person transmission requires close physical exposure to infected blood and other bodily fluids. Moreover, patients suffering from this disease during the period of high infectivity are visibly very ill and too unwell to travel.

In contrast, SARS is emerging in ways that suggest great potential for rapid international spread under the favourable conditions created by a highly mobile, closely interconnected world. Aecdotal data indicate an incubation period of 2 to 10 days (average 2 to 7 days), allowing the infectious agent to be transported, unsuspected and undetected, in a symptomless air traveller from one city in the world to any other city having an international airport. Person-to-person transmission through close contact with respiratory secretions has been demonstrated. The initial symptoms are non-specific and common. The concentration of cases in previously healthy hospital staff and the proportion of patients requiring intensive care are particularly alarming. This "21st century" disease could have other consequences as well. Should SARS continue to spread, the global economic consequences -- already estimated at around USD 30 billion -- could be great in a closely interconnected and interdependent world.

A third collaborative group - clinical, which unites 80 clinicians from 13 countries treating SARS cases, has consistently provided anecdotal information about the lack of efficacy of treatment with specific antibiotics and antiviral agents, and has begun to develop systematic clinical trials of Ribavirin at 2 sites. Their discussions have shed light on features of the disease at presentation, treatment and progression of the disease, prognostic indicators, and discharge criteria. No therapy has been shown to demonstrate any particular effectiveness. The clinicians agreed that a subset of SARS patients, perhaps 10 percent, decline, usually around day 7, and need mechanical assistance to breathe. The care of these people is often complicated by the presence of other diseases. In this group, mortality is high. Age over 40 years also appears to be associated with a more severe form of disease. Countries have made travel recommendations for their citizens, using the guidance provided by WHO and other considerations such as feasibility of medical evacuation of their citizens and their insurance coverage should they become infected.

**Lessons**

Probably the most important lesson learned form this outbreak is the value of innovation and international collaboration. The knowledge obtained in the first 3-week period started 15 Mar 2003 has been remarkable. It demonstrates the value of international cooperation on emerging infections and the importance of early detection and rapid introduction of emergency measures to prevent further international spread and help ensure that imported cases are not allowed to cause disease in others.

When WHO began to set up emergency plans on 15 Mar 2003, identification of the SARS causative agent and the development of diagnostic testing were given paramount importance in the overall containment strategy. Detection of the disease in its early stage, confirmation of cases, understanding modes of transmission, development of protocols for targeted treatment, vaccine research and development, and implementation of disease-specific preventive measures would all depend upon swift progress and results in etiological and diagnostic research. Sound public health measures would also require understanding of the presence and concentration of the pathogen in different tissues and secretions, and patterns of excretion throughout the course of illness and convalescence. So long as the etiological agent remained poorly known, specialists in infectious disease control would be forced to resort to control tools dating back to the "Middle Ages" of microbiology: isolation and quarantine.

Key questions that should be answered in the near future include the exact points during the course of incubation and infection when transmission occurs and whether asymptotic cases are capable of spreading SARS. These questions must be answered in order to evaluate better the extent of SARS spreading, and the success of containment activities.
The SARS response is the rollout of a global alert and response activity under the revision of the International Health Regulations, which provide the legal framework for the surveillance and reporting of infectious disease and for the use of measures to prevent their international spread. SARS is showing how the alert and response activity works in practice for a newly identified disease. It also indicates how the system now in operation could apply to other highly significant infectious disease events, including the next influenza pandemic, the next emerging infection, and the deliberate release of a biological agent in an act of warfare or terrorism.

The scientific community is now contending with an outbreak caused by a new virus. This creates an extra step in the containment response: identification and characterization of the causative agent, which then allows development of a diagnostic test, treatment protocols, and a scientifically sound basis for recommending control measures. This is a step that would not be needed should a biological attack occur using a well-known pathogen such as anthrax or smallpox. The response to an influenza pandemic would likewise not be dealing with an entirely new and poorly understood virus.

The next weeks and months will tell whether the global alert and response will contain the current SARS outbreaks, preventing SARS from becoming yet another endemic infectious disease in human populations, or whether SARS will remain confined to its origins in nature, to re-emerge at yet another time and place. It is clear that the responsibility for containing the emergence of any new infectious disease showing international spread lies on all countries. In a world where all national borders are porous when confronted by a microbial threat, it is in the interest of all populations for countries to share the information they may have as soon as it is available. In so doing, they will allow both near and distant countries -- all neighbours in our globalising world -- to benefit from the understanding they have gained.

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First page - A concise but informative full title of the article. Avoid abbreviations and colloquialisms.

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Third page - footnotes to the title, if any. List of any non-standard abbreviations.

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Number the remaining pages consecutively and type the author’s(s) last name(s) at the top of each page. Write in the first person (except summary) and the active voice whenever possible.

Keep the INTRODUCTION brief, stating clearly the purpose of the article and its relation to other papers on the same subject. Do not give an extensive review of literature.

Provide enough information in the MATERIAL AND METHODS section to enable other investigators to repeat the experiments.

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In the DISCUSSION interpret the results, state their meaning and draw conclusions. Do not simply repeat the results.

Start each section on a separate sheet.
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Footnotes: Avoid footnotes

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The surnames of the authors followed by initials should be given. There should be no punctuation other than commas to separate the authors.

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