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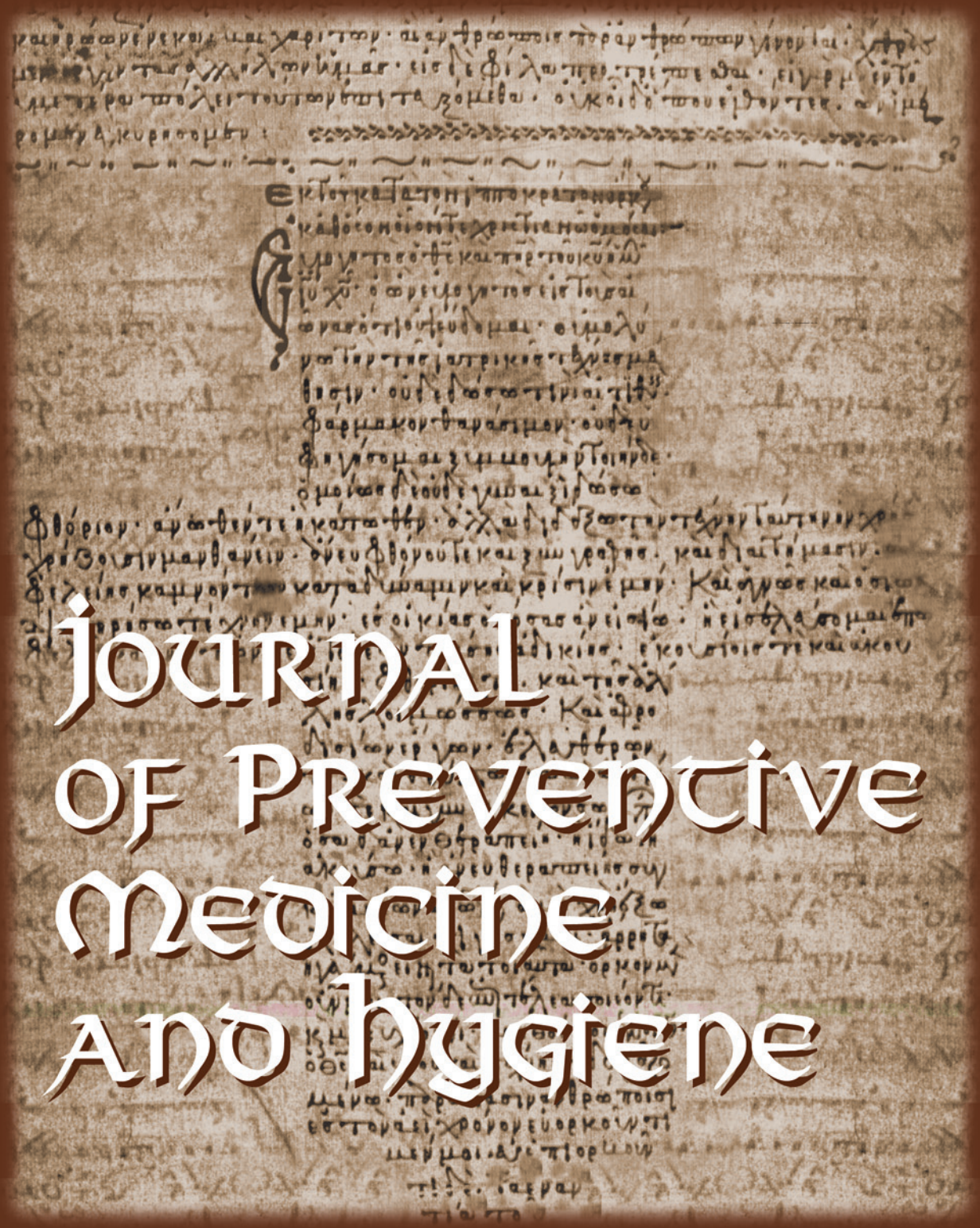

The original document of Hippocrates' oath

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CONTENTS

Original articles

ORIGINAL ARTICLE

Sea buckthorn bud extract displays activity against cell-cultured Influenza virus

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Key words

Sea buckthorn plant extract • Influenza prevention • Antivirals

Summary

Introduction. *Vaccines and antiviral drugs are the most widely used methods of preventing or treating Influenza virus infection. The role of sea buckthorn (SBT) bud dry extract as a natural antiviral drug against Influenza was investigated.*
Methods. *Influenza virus was cultured in the MDCK cell line, with or without SBT bud extract, and virus growth was assessed by HA and TCID50 virus titration in terms of cytopathic effect on cells. Several concentrations of extract were tested, the virus titer being measured on day 4 after infection.*
Results. *After infection, the virus titer in the control sample was calculated to be 2.5 TCID50/ml; treatment with SBT bud extract reduced the virus titer to 2.0 TCID50/ml at 50 µg/ml, while the HA titer was reduced from 1431 (control) to 178. Concentrations lower than 50µg/ml displayed an inhibitory effect in the HA assay, but not in the TCID50 virus titration; however, observation of the viral cultures confirmed a slowdown of viral growth at all concentrations.*
Discussion. *Natural dietary supplements and phytotherapy are a growing market and offer new opportunities for the treatment of several diseases and disorders. These preliminary experiments are the first to show that SBT bud extract is able to reduce the growth of the Influenza A H1N1 virus in vitro at a concentration of 50 µg/ml. This discovery opens up the possibility of using SBT bud extract as a valid weapon against Influenza and, in addition, as the starting-point for the discovery of new drugs.*

Introduction

Influenza is caused by RNA viruses belonging to the family of *Orthomyxoviridae*. Three types of influenza viruses can be distinguished. Among these, influenza A (IAV) viruses mainly infect birds and mammals, whereas influenza B viruses almost only infect humans. Influenza A virus displays constant evolutionary changes, defined as "antigenic drift" [1] and "antigenic shift" [2], enabling new mutant strains to emerge and spread. Influenza viruses constitute a public health problem, as they can give rise to both epidemics and pandemics, causing high rates of morbidity and mortality [1]. Influenza viruses are considered the leading cause of respiratory illness in humans and are responsible for annual seasonal outbreaks that have a serious economic impact [3]. The average global burden of influenza may be in the order of 1 billion cases of flu, 3-5 million cases of severe illness that need hospitalization and 250,000-500,000 deaths yearly [4]. The most effective means of fighting influenza is primary prevention through vaccination [5], which can prevent the disease after exposure or reduce the severity of symptoms. Antiviral drugs can also be used [6]. Indeed, in subjects who are not completely protected by vaccination strategies, an important role has been played by antiviral treatments [7].
Currently, two main classes of antiviral drugs are available; these are based on the ability to block viral M2 ion channels [8] and viral neuraminidase [9]. However, both classes of drugs have been associated with limited efficacy, adverse side effects [1] and the spread of drug resistance in circulating influenza viruses [10, 11]; this last problem has been associated with an uncontrolled administration of the drugs to humans and, sometimes, to farm animals [12]. Other classes of drugs can be used against influenza virus, some of these include: inhibitors of hemagglutinin, endosomes, lysosomes, proteases, polymerases, nucleoprotein (NP), nonstructural-1 (NS1) glycoprotein, RNA synthesis and caspase; however their efficacy in blocking Influenza virus and avoiding the development of antiviral resistances should be further investigated [13]. It is therefore necessary to develop new anti-influenza treatments that are able to inhibit the replication or cellular processes of viruses [1, 7].
Some studies have indicated that certain plant extracts are able to mimic the anti-neuraminidase effect of antiviral drugs. One of these plants is sea buckthorn (SBT) (*Hippophae rhamnoides*): commonly known as sea buckthorn, it belongs to the family Elaeagnaceae [14] and it is a shrubby to bushy plant that grows also at a high altitude and is widely distributed in Eurasia. In India this plant grows predominantly in several high-altitude areas such as Sikkim, Himachal Pradesh, Kashmir, Jammu, and Uttar Pradesh but *Hippophae* is cultivated worldwide for its medicinal properties [15].

All parts of SBT contain large amounts of several active compounds [16] and these include: vitamins (folic acid, vitamin C, vitamin A, vitamin E, vitamin K, riboflavin), carotenoids (lycopene α, β, δ-carotene), phytosterols (amyrins, ergosterol, stigmasterol, lansterol), organic acids (malic and oxalic acids), polyunsaturated fatty acids and essential amino acids [17].

Several medicinal and therapeutic applications utilize the extracts obtained from the leaves of this plant, which display immunomodulatory [18] and anti-inflammatory [19] properties, as demonstrated by Padwad and colleagues [16]. Furthermore, SBT leaves also exert antiviral, anti-bacterial and anti-tumor effects [20, 21]. Preliminary results have also shown that SBT seed extracts have anti-bacterial activity (for example against *Listeria monocytogenes* and *Yersinia enterocolitica*) [22, 23] and anti-viral activities, as reported by the group of Jain against Dengue virus [24].

SBT bud extracts are present in commercial dietary supplements, such as preparations of nutrient and vitamin products [25, 26], and are formulated into Influpirinviral® (PoolPharma). However, no data are currently available on the putative antiviral activity of bud extracts when taken in the form of dietary supplements.

The aim of the present study was to evaluate the therapeutic anti-viral potential of SBT bud extracts on Influenza A/H1N1 virus infection in Madin Darby Canine Kidney cells (MDCK). In 2009, this viral strain was responsible for an Influenza pandemic that spread rapidly around the world [27]. Moreover, it currently circulates in the population, causing seasonal outbreaks, and its antigens are included in the available seasonal influenza vaccines [28].

Methods

Cells and Cell Cultures

Madin Darby Canine Kidney (MDCK) cells were purchased from Sigma-Aldrich (ECACC, Public Health England, Porton Down, United Kingdom) and cultured in Minimum Essential Medium Eagle (EMEM medium) supplemented with 2mM Glutamine, 10% Fetal Bovine Serum (FBS) EU Approved (Euroclone, Pero, Italy) and 100Ul/ml of penicillin-streptomycin. Sub-confluent cultures (70%-80%) were grown at 37°C in a humidified atmosphere containing 5% CO_2, and subcultures were performed every 3-4 days [29]. Except where indicated otherwise, all the above reagents were from Lonza (Verviers, Belgium).

SBT bud dry extract

SBT bud dry extract from PoolPharma S.r.l. (San Giuliano Milanese, Italy) was weighed on a precision scale and dissolved in sterile Dulbecco's Phosphate-Buffered Saline (DPBS) at a final concentration of 1 mg/ml; the pH of the solution was measured (6.96). The dissolved extract was then sterile-filtered through a 0.22 µm filter.

Influenza A/California/7/2009 (H1N1) virus

Influenza A/California/7/2009 (H1N1) virus was obtained from the National Institute for Biological Standards and Control (NIBSC) (Potters Bar, Hertfordshire, United Kingdom) and used according to the instructions provided by the supplier. The virus was propagated in MDCK cells [30], harvested and stored at -80°C. The propagated virus had a tissue culture infectious dose (TCID50) titer of $10^{3.5}$.

Viral growth

Influenza A/California/7/2009 (H1N1) virus was propagated in MDCK cells cultured in UltraMDCK serum-free-medium (SFM) supplemented with 0.5 µg/ml of trypsin from bovine pancreas (TPCK) (Sigma-Aldrich, Saint Louis, MO, USA) and 100 UI/ml of penicillin-streptomycin. MDCK cells were seeded in a T25 cm^2 tissue culture flask at a density of 1 x 10^6 cells/ml. After 24 hours (h), the cell medium was discarded and the cells were washed twice with sterile DPBS. After the DPBS had been discarded from the flask, the cells were treated with 500 µl of virus inoculum (5 ml of solution contained 50 µl of virus at $10^{3.5}$ TCID50/ml (1:100 dilution) and 2.5 µl of TPCK, and incubated for 1 h at 37°C in 5% CO_2. After 1 h, the inoculum was removed, the cells were washed with DPBS, and fresh UltraMDCK SFM, supplemented as previously described, was added. The cells were incubated at 37°C in 5% CO_2 and the cytopathic effect was monitored every day until post-infection day 4. The culture medium was collected and analyzed for TCID50 and hemagglutination titer on the 4^{th} day after infection.

Effect of SBT bud dry extract on MDCK cells

MDCK cells were seeded at a density of 6.5 x 10^5 cells/ml in 6-well plates in complete EMEM medium and were incubated for 24 h at 37°C in 5% CO_2. SBT at different concentrations (1 µg/ml, 5 µg/ml, 10 µg/ml, 30 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml) was then added to the medium in the wells. The cells were checked at 24 h, 48 h and 72 h by means of a light optical microscope to evaluate whether the extract had a cytotoxic effect on them. The experiment was repeated to confirm the preliminary results.

Effect of SBT on viral growth

The viral growth procedure was repeated in the conditions reported above. After infection, the inoculum was removed and the medium was replaced with fresh UltraMDCK SFM (supplemented with 100 UI/ml of penicillin-streptomycin and 0.5 g/ml of TPCK) containing different concentrations of SBT: 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, 30 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml. The infection grade was observed daily for cytopathic effect, and the culture medium was harvested on day 4 to be analyzed for virus content in terms of TCID50 and hemagglutination titer. In parallel, two control flasks were run: the first flask represented the cell control and

the culture medium was added with DPBS; the second flask represented the virus growth control and it was treated only with the live virus.

Virus titration by hemagglutination test

The ability of the influenza virus to agglutinate red blood cells from certain mammalian or avian species can be exploited to check for the presence and hemagglutinating activity of the virus in biological substrates (e.g. serum samples) [31, 32]. To evaluate the hemagglutinating capability of the virus in previously infected cell cultures, the hemagglutination test was used: 100 µl of culture medium from the flask of interest was transferred to the 1st well of 12 of a 96-well V bottom plate then 50 µl of saline solution (0.9% NaCl) (Sigma-Aldrich, Saint Louis, MO, USA) was added from well 2 up to well 12; 2-fold serial dilutions of the culture medium (contained in the 1st well) in the saline solution (wells 2-12) were performed from the 1st well up to the 12th well.
Then, 50 µl of turkey red blood cell (RBC) suspension (0.5% in saline solution) (Emozoo Snc, Casole d'Elsa, Italy) was added to each well and the plate was incubated for 45 minutes at room temperature (RT). After incubation, the plate was tilted to allow non-hemagglutinated RBCs to drip from the bottom of the wells and the result was read; the reciprocal of the highest dilution of the culture medium that was still able to cause agglutination indicated the titer of the virus in the culture medium. The experiment was repeated, this time with a 1:10 starting dilution of the culture media, and the virus titer was calculated in terms of hemagglutinating units (HAU) in 1 ml by applying the following formula: HAU in 1 ml=$20x10^{[LOG(Dilution1)+LOG(Dilution2)]/2}$.

Virus titration by $TCID_{50}$

The "TCID50" titer is the viral dose that gives rise to a cytopathic effect in 50% of cells in the inoculated culture. The virus titer was determined by means of the TCID50 assay, using the Spearman/Karber method [33] on treated MDCK cells, as reported by Lugovtsev [34]. Twelve plastic tubes (1.5 ml) were prepared and loaded with 900 µl of cell medium (EMEM), except for the 11th tube; 100 µl of supernatants from cultures was then transferred into the first tube and serial 10-fold dilutions were performed from tube 1 to up to tube 10. The contents of the tubes were transferred into a 96-well cell culture plate: the content of the 1st tube was transferred into the 1st column of the plate, the content of the 2nd tube was transferred into the 2nd column, and so on up to the 10th tube. The 11th column was left empty. At the end of the dilution steps, 100 µl of cell suspension (in complete EMEM medium, with 0.5% FBS and 0.5 µg/ml of TPCK, at a cell density of 5 x 10^5 cells/ml) was added to each well. The 12th column, containing only cell medium and cell suspension, was used as a cell control. The plates were incubated for 5 days and the TCID50 titer was evaluated by checking the cytopathic effect in the cell mono-layer by means of a light microscope. The results were calculated by applying the Spearman/Karber formula [33]: TCID50/100µl= X_0-d/2+d(ΣXi/n), where X_0 is the positive logarithm of the highest dilution at which all wells are positive for cytopathic effect, d represents the dose distance in log, *n* is the number of repeats per dilution and ΣXi is the sum of all positive wells, starting from X_0. In the case of 10-fold dilution, d=$\log_{10}10$=1 and the formula can be simplified to TCID50/100µl= X_0-1/2+(ΣXi/n). To express the results as TCID50/ml, one log was added.

Results

Evaluation of the cytotoxic effect of SBT on MDCK Cells

The possible cytotoxic effect of SBT on MDCK cells previously infected with Influenza A H1N1 virus was evaluated by means of direct observation of cells under a light optical microscope. SBT treatment of MDCK cells showed no cytotoxic effect up to a concentration of 50 µg/ml. At 75 µg/ml and 100 µg/ml, SBT had a cytotoxic effect on the cells: at 75 µg/ml, the cell monolayer had a discontinuous appearance and floating cells were present in the culture medium; this effect was more evident at a concentration of 100 µg/ml (Tab. I).

Tab. I. Evaluation of the cytotoxic effect of SBT on MDCK Cells. This table shows the effect of different concentrations of SBT on MDCK cell cultures after 72 h of incubation. The concentrations of SBT tested were: 1 µg/ml, 5 µg/ml, 10 µg/ml, 30 µg/ml, 50 µg/ml, 75 µg/ml and 100µg/ml. No toxic effect was observed up to a concentration of 75 µg/ml. At 75 µg/ml and 100 µg/ml of SBT, the cells in the wells showed signs of toxicity: discontinuous cell layer and floating cells in the medium.

SBT concentration	Effect
Cell control	No effect
1 µg/ml	No effect
5 µg/ml	No effect
10 µg/ml	No effect
30 µg/ml	No effect
50 µg/ml	No effect
75 µg/ml	Discontinuous cell layer, floating cells
100 µg/ml	Discontinuous cell layer, floating cells

SBT effect on viral growth results

Hemagglutination titer results

The ability of SBT bud extract to reduce Influenza H1N1 viral growth was evaluated on post-infection day 4. MDCK cell culture supernatants were harvested from the culture plates and assayed for hemagglutination titer. Concentrations of SBT ranging from 2.5 µg/ml to 50 µg/ml markedly reduced the hemagglutination titer from 28621.6 HAU/ml (the value obtained from the viral growth control sample) (Fig. 1). The two highest concentrations of SBT used (75 µg/ml and 100 µg/ml) completely inhibited viral growth. However, they dis-

Fig. 1. Hemagglutination Titration results on day 4 after SBT treatment. Supernatants of viral cultures treated with SBT were analyzed by means of hemagglutination assay to determine whether the treatment was able to reduce the spread of Influenza virus. The values of the hemagglutination titer in 1 ml (volume assayed) are shown on the y axis; the x axis reports the concentrations of SBT (2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, 30 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml) used to treat viral cultures. No SBT was added to the virus control sample. The control sample displayed a titer of 28636; treatment with SBT dramatically decreased the hemagglutination titer. The most effective concentration of SBT was 50 µg/ml. At 75µg/ml and 100µg/ml, SBT totally inhibited viral growth, but in these cases the results could be affected by SBT toxicity. These results represent a mean of two experiments *(n = 2)*.

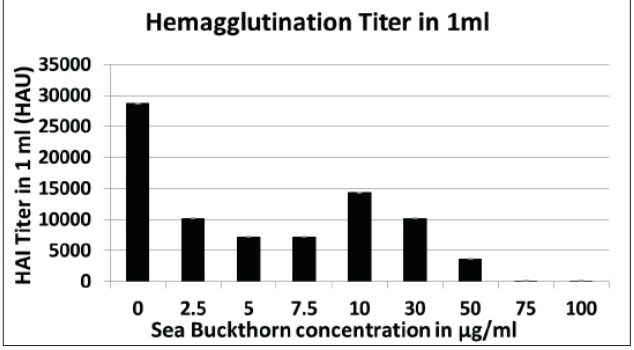
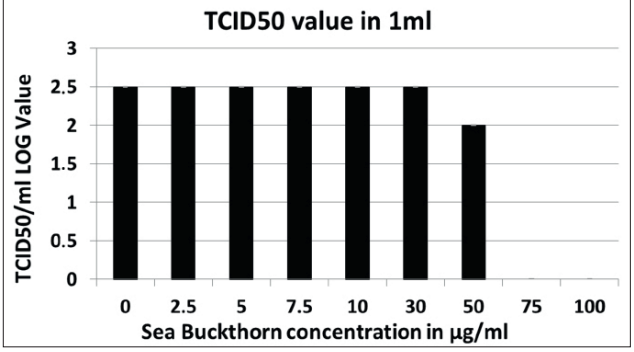


Fig. 2. TCID50 Virus titration results. The graph shows the results of the TCID50 titration assay performed on supernatants from viral MDCK cultures treated with SBT. The values of the TCID50 titer in 1 ml are shown on the y axis; on the x axis the concentrations of SBT used to treat viral cultures are reported: 0 µg/ml (virus control), 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, 30 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml. The virus control displayed a titer of 102.5 TCID50; the TCID50 titer was reduced when SBT was used at a concentration of 50 µg/ml. At 75 µg/ml and 100 µg/ml, SBT inhibited viral growth totally. These results represent a mean of two experiments *(n = 2)*.

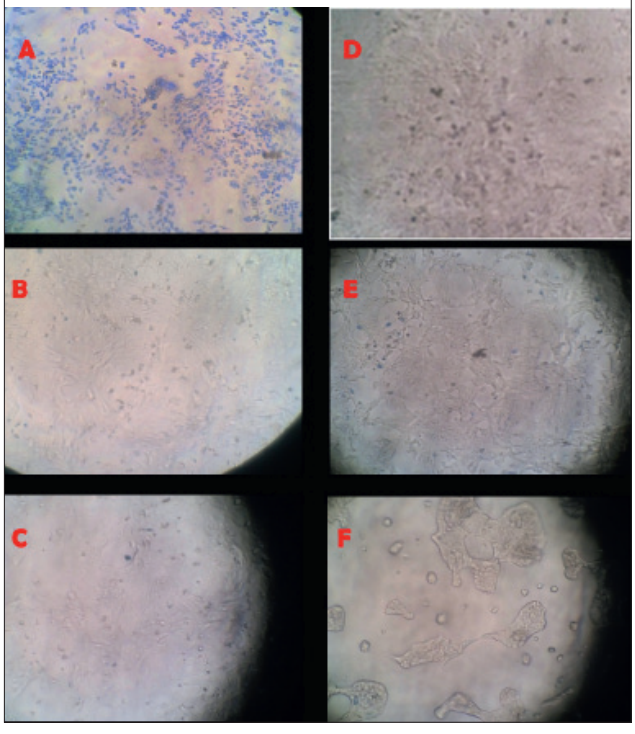


played a toxic effect on the cells; the results obtained at these high concentrations may therefore be affected cell toxicity, as was observed after staining the cell cultures with Trypan blue.

TCID50 virus titration results

Cell culture supernatants were harvested on day 4 after infection and treatment and assayed for TCID50 virus titer to assess the number of infectious (live) viral particles released from the infected cells in the culture medium. The cytopathic effect was evaluated on day 5. The treatment of viral cultures with SBT at a concentration of 50 µg/ml markedly reduced the viral titer in terms of TCID50 (Fig. 2). At 75 µg/ml and 100 µg/ml, SBT was able to inhibit viral growth completely; however, this value could be affected by the toxicity that SBT showed on cells at such high concentrations. Direct observation of the viral cultures confirmed the results obtained from TCID50 and hemagglutination titration. The concentration of SBT that proved to have the greatest impact on viral growth while exerting little negative effect on cells was 50 µg/ml. Figure 3 reports a series of representative pictures showing the effect of SBT at 0, 10, 30, 50, 75 and 100 µg/ml on day 4 on MDCK cells infected with Influenza A/H1N1 virus.

Fig. 3. Cell viability of MDCK cells infected with Influenza H1N1 upon treatment with SBT. The figure shows a series of representative pictures from the light optical microscope (Trypan blue-stained; original magnification 100X) showing MDCK cell cultures infected with Influenza virus H1N1 A/California/7/2009 and treated with different concentrations of SBT: A, virus control; B, 10 µg/ml; C, 30 µg/ml; D, 50 µg/ml; E, 75 µg/ml; F, 100 µg/ml. Cells were stained with Trypan blue to observe dead cells. The virus control displayed a large number of dead cells (blue spots); 10 µg/ml and 30 µg/ml of SBT induced a reduction in the number of dead cells, and the cellular monolayer was more compact. Picture D represents the 50 µg/ml SBT concentration: no cytopathic effect and a limited number of dead cells. The 75 µg/ml and 100 µg/ml SBT concentrations showed a discontinuous cell layer with many dead cells in the culture medium.



Discussion

In recent years, since the spread of resistant viral strain towards the available pharmacologic treatments and the occurrence of unpredictable pandemics, many investigations have been carried out on new computer-designed molecules or available compounds in a search for new

anti-influenza drugs. In the present study, the antiviral activity of SBT bud extract (contained in Influpirinviral®) against influenza H1N1 A/California/7/2009 was evaluated *in vitro* on MDCK cells infected with this virus. A previous study conducted by Jain and colleagues [24] demonstrated that SBT leaf extract at a concentration of 50 µg/ml exerted an antiviral activity against Dengue virus. We found that SBT bud extract had an antiviral activity on influenza virus H1N1, especially at 50 µg/ml (the same concentration used by Jain's group). Specifically, after 4 days the viral titer was evaluated in terms of TCID50 titer and hemagglutination titer upon treatment with SBT bud extract; both methods revealed that, at 50 µg/ml, the viral titer was reduced in comparison with the virus control. Direct observation of the virus cultures confirmed the antiviral activity at 50 µg/ml, although an antiviral effect was also visible at lower concentrations, starting from 2.5 µg/ml. When SBT was used at high concentrations (75 µg/ml and 100 µg/ml), viral growth was completely inhibited. However, the treatment had an adverse effect on cell cultures, and this could have affected the results obtained at these concentrations.

Conclusions

The data obtained from this preliminary study confirmed that SBT bud extract has an antiviral activity on influenza H1N1 A/California/7/2009 *in vitro,* supporting its potential use as an anti-influenza agent. Nevertheless, further investigations are needed in order to generate more data, to evaluate the preventive role of SBT treatment against Influenza infection, and to understand the specific mechanism of action of this extract both *in vitro* and *in vivo*.

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■ Conflicts of interests: Dr. Brenno Canovi is the Scientific Director of Pool Pharma, the company that distributes the Influpirinviral®, the dietary supplement containing sea buckthorn bud extract.

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ORIGINAL ARTICLE

A novel multiplex one-step real-time RT-PCR assay for the simultaneous identification of enterovirus and parechovirus in clinical fecal samples

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Key words

Enterovirus • Parechovirus • Multiplex one-step real-time RT-PCR

Summary

Introduction. *Enterovirus (EV) and parechovirus (PeV) can either infect humans asymptomatically or can cause gastroenteritis, respiratory symptoms and, sometimes, severe disease. As the number of newly identified EV and PeV genotypes keeps increasing, diagnostic methods need to be updated. To this end, we described a novel multiplex one-step real-time RT-PCR to detect EV and human PeV (HPeV) simultaneously in fecal samples collected from children with rotavirus group A (RV-A)-related gastroenteritis.*
Methods. *The specificity and sensitivity of the EV/HPeV real-time RT-PCR were evaluated with two 2011 Quality Control for Molecular Diagnostics (QCMD) panels for EV and HPeV detection. RNA was extracted from 111 RV-A-positive fecal samples collected from children up to 5 years of age who had been hospitalized for gastroenteritis from September 2010 to August 2011.*
Results. *The EV/HPeV real-time RT-PCR showed a 100% sensitivity and specificity for EV and 91% and 91.7% for HPeV, respectively. Of the 111 RV-A-positive stool specimens, 28 (25.2%) were EV-positive and 7 (6.3%) were HPeV-positive. No clinical differences between children with single or double infections were observed.*
Discussion. *In our study, the frequency of EV and HPeV infections was surprisingly high, thus underlining the importance of including EV and HPeV detection in diagnostic panels. The multiplex real-time RT-PCR presented in this paper can therefore be a useful method in a diagnostic setting.*

Introduction

The *Picornaviridae* family currently consists of 46 species grouped into 26 genera [1]. Three of these genera include human pathogens: Enterovirus (EV), Hepatovirus (hepatitis A virus), and Parechovirus (PeV). EVs and human PeVs (HPeV) may be associated with gastroenteritis as well as upper and lower respiratory tract infections [2-4], and can be detected in fecal and respiratory samples [5-7]. Both viruses have tropism for neuronal cells, therefore implying their involvement in aseptic meningitis, encephalitis, encephalomyelitis [3, 8] and white matter abnormalities [9, 10].
In the last few years, advances in metagenomics and molecular biology have enabled the in-depth analysis of picornaviruses, the discovery of new EV and PeV genotypes, as well as a continuous update of their taxonomy [11]. The enterovirus genus now consists of 12 species and contains more than 100 genotypes: human EVs (A-D), non-human EVs (E-H and J), and rhinoviruses A-C - the most recently categorized within the EV genus [12]. The parechovirus genus consists of two species: zoonotic Ljungan virus and HPeV, the latter containing 16 genotypes [1]. As the number of genotypes increases, their molecular detection methods need to be updated in order to ensure high sensitivity and specificity, particularly in a diagnostic setting.

This paper presents the novel multiplex one-step real-time RT-PCR assay for the simultaneous detection of EV and HPeV for clinical fecal samples collected from hospitalized children with acute gastroenteritis in Lombardy (northern Italy) from September 2010 to August 2011.

Methods

Multiplex one-step real-time RT-PCR assay

The assay was performed by using the following primer/probe sets: 5'-GGTGCAAGAGTCTATTGAGC-3' (EV-08 forward), 5'-CACCCAAGTAGTCGGTTCC-3' (EV-08 reverse), and 5'-CCGGCCCCTGAATG-3' (EV-probe) specific for the 5'non-translated region (5'-NTR) (nt. 415-555) of EVs, as published by Nielsen et al. [13]; 5'-GTAACASWWGCCTCTGGGSCCAAAAG-3' (AN345-forward), 5'-GGCCCCWGRTCAGATCCAYAGT-3' (AN344-reverse), and 5'-CCTRYGGGTACCTYCWGGGCATCCTTC-3' (AN257-probe) specific for the 5'-NTR (nt. 421-615) of HPeVs as published by Nix et al. [14]. The following two fluorescent dyes were used: 6-FAM for the EV-probe and VIC for the HPeV-probe. BHQ1 quencher was used for both probes.

The multiplex one-step real-time RT-PCR for EV and HPeV detection was prepared with 5 µl of RNA in a total volume of 25 µl with the AgPath-ID One-Step RT-PCR kit (Ambion®, Life Technologies, USA). The reaction mixture contained 1 µM of each EV primer, 0.4 µM of each HPeV primer and 0.2 µM of each probe and was carried out in a 7300 Real-time PCR System (Applied Biosystem®, Life Technologies, USA) with the following thermal profile: 50 °C × 30 min, 95 °C × 15 min, and 50 cycles at 95 °C × 15 sec, 58 °C × 30 sec, and 72 °C × 10 sec.

Sensitivity and specificity of the multiplex one-step real-time RT-PCR

The sensitivity and specificity of the multiplex one-step real-time RT-PCR were evaluated with two Quality Control for Molecular Diagnostics (QCMD) panels [15]: the QCMD 2011 Enterovirus RNA EQA Programme (2011 EV-QCMD), and the QCMD 2011 Parechovirus RNA EQA Programme (2011 PeV-QCMD). The 2011 EV–QCMD panel consisted of 12 samples: 10 were EV-positive (Coxsackievirus A16, A21, and A24; EV68; EV71; Echovirus 11 and 30; stock dilutions: 10^{-3}-10^{-7}), one contained human Rhinovirus 16, and one was viral transport medium (VTM). The 2011 PeV-QCMD panel consisted of 11 samples: 8 were HPeV-positive (HPeV 1-5; stock dilutions: 10^{-3}-10^{-7}), one contained human Rhinovirus 16, one contained Coxsackievirus A21, and one was VTM.

The inter- and intra-assay variation of the one-step real-time RT-PCR were calculated and expressed by coefficient of variation (%CV).

Clinical fecal specimens

From September 2010 to August 2011, fecal samples were collected from children aged 0-5 years old (62.2% males, median age: 12 months; inter-quartile range [IQR]: 12 months) who had been hospitalized for acute rotavirus-A related (RV-A) gastroenteritis, in Lombardy (northern Italy). Rotavirus infection was diagnosed on admission to hospital by commercial routine immunochromatographic or latex-agglutination tests. Overall, in this study, 111 RV-A-positive fecal samples were analyzed anonymously by the multiplex one-step real-time RT-PCR method described previously.

At the time of hospitalization, all children presented with clinical signs of gastroenteritis, i.e. fever and/or abdominal pain and/or diarrhea and/or vomiting. Diarrhea was the most frequently reported symptom (88/111: 79.3%), followed by vomiting (78/111: 70.3%) and fever (67/111: 60.4%). Abdominal pain was detected in 38 cases (34.2%) and only in association with one or more symptoms. The majority of children (84/111: 75.7%) presented with more than one gastroenteritis sign and all symptoms were present in 20.6% (23/111) of children studied.

RNA was extracted for testing with the commercial kit Invisorb® Spin Virus RNA Mini kit, (Stratec Molecular, Germany). To monitor RNA extraction each clinical sample was tested for the presence of human RNase P gene (RNP) by using a specific primer/probe set (TaqMan® RNase P Assay, ABY® dye/QSY® probe, Life Technologies, USA). Five µl of each sample's RNA were added to a reaction mixture that consisted of 12.5 µl AgPath-ID One-Step RT-PCR kit (Ambion®, Life Technologies, USA), 0.4 µM of each RNase P primer, 0.2 µM of probe and biological grade water up to 20 µl. The reaction was carried out at the same conditions of the multiplex one-step real-time RT-PCR, as described above. Each sample should exhibit RNP reaction curves that cross the threshold line at or before 40 cycle threshold (C_T). A sample was considered positive to EV/HPeV when a curve crosses the threshold before or at 40 C_T.

Results

Sensitivity and specificity of the multiplex one-step real-time RT-PCR assay

The EV/HPeV real-time RT-PCR assay presented in this study detected all EV-positive samples included in the 2011 EV-QCMD and 2011 PeV-QCMD panels (sensitivity: 100%). The assay detected all EV-dilutions until the least concentrated (limit of detection: 10^{-7}), which were identified by 55.4% of all laboratories participating in the 2011 EV-QCMD programme. Therefore, the EV/HPeV real-time RT-PCR detected all EV species included in 2011 EV- and PeV-QCMD panels and no cross-reactions were observed with the rhinoviruses included in the panels: the assay specificity was 100%.

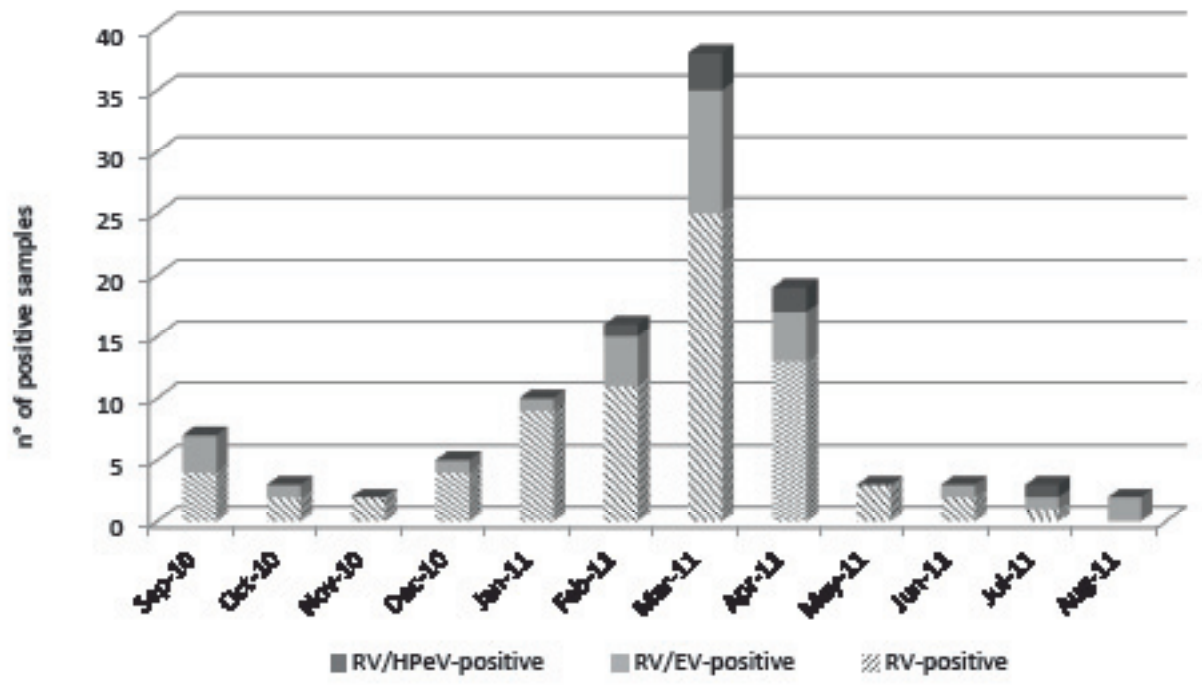
Our multiplex assay identified all HPeV-positive specimens included in the 2011 PeV-QCMD panel except the PeV3 10^{-7} stock dilution, which was detected successfully only by 24.1% of all participants in the 2011 PeV-QCMD programme. The limit of detection for HPeV was 10^{-6} and the assay sensitivity was 91%. The specificity of the multiplex was 91.7% for HPeV, because a cross-reaction between HPeV and Coxsackievirus A16 was identified. A singleplex assay for HPeV was set up with the same conditions as the multiplex assay and no differences in sensitivity and specificity were observed.

No differences in terms of C_T values (mean standard deviation: 0.97 for EV and 0.59 for HPeV) were observed when mixes of EV and HPeV concentrations were used. The intra-assay %CVs of the real-time RT-PCR assay were 0.99% and 2.09% for EV and HPeV, respectively. The inter-assay %CV was 4.1% for EV and 3.9% for HPeV.

EV and HPeV detection in clinical fecal specimens

During the 12-month study period, 111 RV-A-positive stool samples were collected and analyzed with the multiplex assay. Twenty-eight (28/111: 25.2%) were positive for EV and 7 (7/111: 6.3%) for HPeV. No triple infections (EV/HPeV/RV-A) were detected.

Fig. 1. Number of positive samples for EV, HPeV and RV–A in children ≤5 years hospitalized with gastroenteritis from September 2010 to August 2011.



The median C_T values of EV-positive samples was 34.31 (IQR: 2.70; range: 18.22-39.75); the median C_T values of HPeV-positive samples was 36.66 (IQR: 1.01; range: 28.25-39.02).

Figure 1 displays the monthly trend of RV-A-positive specimens with the detection of EV and HPeV. The occurrence of RV-A infections peaked in late winter/beginning of spring (March 2011). RV-A/EV co-infections were found throughout the year, especially (10/28: 35.7%) during springtime (March-April 2011). RV-A/HPeV co-infections were observed from February to April and in July 2011.

In our study, the mean age of children with co-infection was 19 months for RV-A/EV (range: 26 days – 60 months; median age: 12 months; IQR: 12 months) and 23.3 months for RV-A/HPeV (range: 5 – 48 months; median age: 24 months; IQR: 6 months). However, no significant differences (p-value>0.05) were found between the two groups. In addition, patients with a double infection did not show more severe clinical symptoms.

Discussion

As the number of novel picornaviruses identified over the last few years increases considerably, the continuous validation and update of existing EV and HPeV assays is essential.

We set up a multiplex one-step real-time RT-PCR assay combining the EV assay by Nielsen et al. [13] and the HPeV assay by Nix et al. [14]. The sensitivity and the specificity of the EV/HPeV real-time RT-PCR were tested on two 2011 EV/PeV QCMD panels and resulted with 100% for EV and 91% and 91.7% for HPeV, respectively. Contrary to another study [16], it is noteworthy that no cross-reactions between EVs and other viruses, such as rhinoviruses, were observed here.

Viral intestinal infections are the most common cause of acute infectious diarrhea in children [17]. Over the past decade, there have been major advances in the understanding of viral gastroenteritis etiology. Group A rotavirus is responsible for the majority of acute diarrhea in young children worldwide [18]. Furthermore, other viruses like norovirus, adenovirus, enterovirus, bocavirus, sapovirus, astrovirus, calicivirus, and, more recently, torovirus and parechovirus have also been identified thanks to the development of rapid molecular techniques [18, 19]. The frequencies of EV (about 25%) and HPeV (about 6%) infections observed in our study were much higher than those reported by Rovida et al. [20], who showed the frequencies of EV and HPeV infections in pediatric and adult populations with gastroenteritis to be 3% and 1%, respectively. Several studies have evaluated the clinical impact of mixed infections believed to be the cause of severe diarrhea in children under 5, and they have found that the frequencies of mixed infections fluctuate between 5% and 34% [19-23]. It is difficult to compare such findings as dual infections are often misdiagnosed or not investigated at all in routine laboratory work. For example, Rimoldi et al. [24] only included EV in their viral gastroenteritis panel and no samples were EV-positive, whereas HPeV was not considered at all.

Conclusions

The impact of EV and HPeV on childhood gastroenteritis is still unknown due to the lack of systematic testing of clinical samples. In our study, the high frequency of both EV and HPeV in clinical fecal samples underlines the importance of introducing EV and HPeV assays in routine laboratory practice.
A fast, sensitive and specific test, such as the EV/HPeV multiplex real-time RT-PCR described above, is a useful tool for the detection of these viruses in fecal samples as well as in other clinical samples.

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ORIGINAL ARTICLE

Emergence of plasmid-mediated quinolone-resistant determinants in *Klebsiella pneumoniae* isolates from Tehran and Qazvin provinces, Iran

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Key words

Klebsiella pneumoniae • Quinolones resistance • *qnr*

Summary

Background. *Plasmid-mediated quinolone resistance is an increasing clinical concern, globally. The major objective of the present study was to identify the qnr-encoding genes among the quinolone non-susceptible K. pneumoniae isolates obtained from two provinces in Iran.*
Methods. *A total of 200 K. pneumoniae isolates were obtained from hospitals of Qazvin and Tehran, Iran. The identification of bacterial isolates was carried out by standard laboratory methods and API 20E strips. Susceptibility to quinolone compounds were examined by standard Kirby-Bauer disk diffusion method according to the CLSI guideline. PCR and sequencing were employed to detect qnrA, qnrB and qnrS-encoding genes.*
Results. *Of 200 K. pneumoniae isolates, 124 (62%) were non-susceptible to quinolone compounds among those 66 (53.2%) and 58 (46.8%) isolates showed high and low-level quinolone resistance rates, respectively. Out of 124 quinolone non-susceptible isolates, qnr-encoding genes were present in 49 (39.5%) isolates with qnrB1 (30.6%) as the most dominant gene followed by qnrB4 (9.7%), and qnrS1 (1.6%) either alone or in combination.*
Conclusions. *This study, for the first time, revealed the high appearance of qnrB1, qnrS1 and qnrB4 genes among the clinical isolates of K. pneumoniae in Iran. Therefore, the application of proper infection control measures and well-established antibiotic administration guideline should be strictly considered within our medical centers.*

Introduction

Klebsiella pneumoniae (K. pneumoniae) is an opportunistic pathogen causing several nosocomial infections such as urinary tract infections, pneumonia, septicemia, and soft tissue infections [1]. This organism is also known as a community-acquired potential pathogen [2]. Health care associated infection caused by this organism has been linked to high mortality and morbidity especially among the patients admitted to intensive care units [3, 4].
Quinolones are a group of synthetic antibacterial agents that are widely used in routine clinical practice [5]. The new quinolones compounds (6-fluoroquinolones) exhibit broad spectrum of antibacterial activity against Gram-negative, mycobacterial pathogens, and anaerobes. Moreover, these agents show a good-to-moderate oral absorption and tissue penetration with favorable pharmacokinetics in humans, creating desirable clinical efficacy in treating many kinds of infections [6, 7]. Quinolones inhibit the function of bacterial DNA gyrase and topoisomerase IV [8]. While the first and second generation fluoroquinolones selectively inhibit the topoisomerase II ligase domain or DNA gyrase activity, the quinolones of third and fourth generations are with more tendency for topoisomerase IV ligase [9]. Excessive and inappropriate administration of antimicrobial agents such as quinolones has increased the emergence of multidrug resistant *K. pneumoniae* isolates which makes the process of antimicrobial therapy to become marginal and problematic [10, 11]. In recent years, several studies have demonstrated that the appearance of quinolone-resistant *K. pneumoniae* is rising at a faster rate, worldwide [12-15]. Infections caused by resistant organisms are often due to extensive cross-resistance with other antimicrobials, including beta-lactams and aminoglycosides [16]. Quinolone resistance in *Enterobacteriaceae* mainly occurs through chromosomal mutations in the genes coding for DNA gyrase and topoisomerase IV, changes in outer membrane and efflux proteins or in their regulatory mechanisms [17]. Findings from recent studies show that plasmid-mediated resistance, associated with the pentapeptide proteins of the *qnr* family, might play a crucial role in quinolone compound resistance [18]. Three major groups of *qnr* determinants, *qnrA*, *qnrB*, and *qnrS*, are increasingly being identified in the clinical isolates of various enterobacterial species, worldwide [19]. It was in 1998 that the first plasmid-mediated quinolone resistance determinant, *qnrA*, was reported in a *Klebsiella pneumoniae* strain from the United States [20]. Since then two *qnr* determinants, *qnrB* and *qnrS* have been discovered in other *Enterobacte-*

riaceae species such as *Citrobacter Koseri*, *Escherichia coli*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* from Asia and Europe [21-24]. To date, there has been no report for the frequency of *qnr* genes among *K. pneumoniae* isolates in Iran. In the current study, for the first time, we described the frequency of *qnr* determinants (*qnrA, qnrB,* and *qnrS*) among the isolates of quinolone non-susceptible *K. pneumoniae* collected from hospitals of Qazvin and Tehran provinces.

Methods

Bacterial isolates and Antimicrobial susceptibility

In this descriptive study, a total of 200 clinical isolates of *K. pneumoniae* were collected from hospitalized patients in several teaching hospitals in Tehran and Qazvin during 2012-2013. The isolates were obtained from different clinical specimens including urine, wound, trachea, secretions, blood, and ascites. All isolates were identified by standard laboratory methods and confirmed with the API 20 E (bioMérieux, France) strips. All isolates were kept at -70°C in trypticase soy broth containing 20% glycerol and subcultured twice before testing. The mean age of patients (77 (38.5%) male and 123 (61.5%) female) was 51.7±17.4 (range17-83) years. Written informed consent was obtained from all subjects enrolled in this study. Kirby-Bauer disk diffusion technique was performed according to the CLSI guideline to identify quinolone resistance using nalidixic acid (30µg), ciprofloxacin (5 µg), gatifloxacin (5 µg), norfloxacin (10 µg), and levofloxacin (5 µg) disks [25]. In this study the isolates were classified either as high-level quinolone resistant if the resistance to both nalidixic acid and ciprofloxacin disks was observed or low-level quinolone resistant in the cases of resistance to nalidixic acid, presence of intermediate isolates or ciprofloxacin-susceptible organisms [26]. Antibiotic disks were purchased from the Mast (Mast Diagnostics Group Ltd, Merseyside, UK). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains in antimicrobial susceptibility testing.

Detection of *qnr* determinants

Detection of *qnrA*, *qnrB,* and *qnrS* plasmid-mediated quinolone resistance genes was performed using PCR and specific primers (Tab. I). Plasmid DNA was extracted by plasmid mini extraction kit (Bioneer Company, South Korea). PCR amplifications were applied in a thermocycler (Applied Biosystems, USA) as follows: 95ºC for 5min and 35 cycles of 1min at 95ºC, 1min at specific annealing temperature for each primer and 1min at 72ºC. A final extension step of 10 min at 72ºC was performed. Amplification reactions were prepared in a total volume of 25µl (24µl of PCR master mix plus 1µl of template DNA) including 5ng of genomic DNA, 2.0U of Taq DNA polymerase, 10mM dNTP mix at a final concentration of 0.2mM, 50mM MgCl2 at a final concentration of 1.5mM, 1µM of each primer, and 1X PCR buffer (final concentration). PCR products were electrophoresed on 1% agarose gel at 100 volts and later stained with ethidium bromide solution and finally visualized in a gel documentation system (UVtec, UK). The purified PCR products were sequenced by the Macrogen Company (Seoul, South Korea) and the sequence alignment and analysis were performed online using the BLAST program of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Data were summarized using mean ± SD (standard deviation), proportional frequency and confidence interval for microbiological, clinical, and demographic characteristics. All analyses were carried out using a Statistical Software Package, SPSS for windows version 16.0 (Chicago, IL, USA).

Results

In this study, the bacterial isolates were recovered from different clinical specimens including urine (110-55.0%), trachea (59-29.5%), wound (18-9.0%), blood (8-4%), and ascites (5-2.5%). These isolates were obtained from the patients admitted to intensive care units (96-48.0%), internal medicine (54-27.0%), infectious diseases (35-17.5%), surgery (13-6.5%), and orthopaedic (2-1.0%) wards. The results of antimicrobial susceptibility testing showed the resistance rates against the antimicrobial agents used in our study varied between 20% and 58%. Overall, nalidixic acid (58%) and ciprofloxacin (34.5%) revealed the highest rates of resistance among the antimicrobials tested whereas levofloxacin and norfloxacin also demonstrated high susceptibility rates of 80% and 77%, respectively (Tab. II). In total,

Tab. I. The primers used for detection of qnr genes in K. pneumoniae isolated from Qazvin and Tehran hospitals.

PCR targets	Primer sequence (5′-3′)	Annealing temperatures (ºC)	References
qnrA1–6	F: ACGCCAGGATTTGAGTGAC R: CCAGGCACAGATCTTGAC	49	27
qnrB1-3, 5, 6, 8	F: GGCACTGAATTTATCGGC R: TCCGAATTGGTCAGATCG	49	27
qnrB4	F: AGTTGTGATCTCTCCATGGC R: CGGATATCTAAATCGCCCAG	53	27
qnrS1–2	F: CCTACAATCATACATATCGGC R: GCTTCGAGAATCAGTTCTTGC	53	27

Tab. II. Antibiotic susceptibility of *K. pneumoniae* against quinolone compounds.

Antimicrobial agents	Resistance n (%) [CI]	Intermediate n (%) [CI]	Susceptible n (%) [CI]
Nalidixic acid	83(41.5) [34.7-48.3]	33(16.5) [11.4-21.6]	84(42) [35.2-48.8]
Ciprofloxacin	53(27) [20.8-33.2]	15(7.5) [3.8-11.2]	131(65.5) [58.9-72.1]
Gatifloxacin	39(19.5) [14-25]	27(13.5) [8.8-18.2]	134(67) [60.5-73.5]
Norfloxacin	37(18.5) [13.1-23.9]	9(4.5) [1.6-7.4]	154(77) [71.2-82.8]
Levofloxacin	36(18) [12.7-23.3]	4(2) [0.1-3.9]	160(80) [74.5-85.5]

CI = 95% Confidence interval

66 (53.2%) and 58 (46.8%) of isolates showed high and low-level quinolone resistance, respectively.

PCR and sequencing showed the presence of *qnr*-encoding genes in 49 (39.5%) of quinolone non-susceptible *K. pneumoniae* isolates among those *qnrB1* (38-30.6%) was the most common gene followed by *qnrB4* (12-9.7%) and *qnrS1* (2-1.6%) genes either alone or in combination. The study isolates were negative for *qnrA* gene. As shown in Table III, *qnrB1* was found to coexist with *qnrB4* in 3 (2.4%) isolates. Overall, 25 (37.9%) high level quinolone resistant isolates carried *qnr* genes in which 19 (28.8%), 4 (6.1%), and 2 (3%) isolates carried *qnrB1*, *qnrB4,* and *qnrS1* genes, respectively. In addition, 24 (41.4%) low level quinolone resistant isolates were positive for *qnr* genes among those 19 (32.8%) isolates carried *qnrB1* gene followed by *qnrB4* in 8 (13.8%) isolates. Among the high and low-level quinolone resistance isolates, *qnrB1 was the* most frequent gene compared to other genes. Table IV shows that *qnr*-positive isolates were mostly recovered from urine (42.9%) followed by trachea secretion (36.7%) samples. The patients affected by these organisms were mostly admitted to ICU (59.2%) and internal medicine (20.4%) wards.

Tab. III. Distribution of *qnrB1, qnrB4,* and *qnrS1* genes among *qnr*-positive *K. pneumoniae* isolates.

qnr-encoding genes	N of isolates n (%) [CI]
qnrB1	35 (28.2%) [20.3-36.1]
qnrB4	9 (7.3%) [2.7-11.9]
qnrS1	2 (1.6%) [0-3.8]
qnrB1+qnrB4	3 (2.4%) [0-5.1]
qnr negative	75(60.5%) [51.9-69.1]
Total	124 (100%)

CI: 95% Confidence interval

Tab. IV. Frequency of qnr-positive K. pneumoniae isolates based on hospital wards and source of Specimens (n = 49).

Wards	N° of isolates n (%) [CI]	Specimens	N° of isolates n (%) [CI]
ICU	29 (59.2%) [45.4-73]	Urine	21 (42.9%) [29-56.8]
Internal medicine	10 (20.4%) [9.1-31.7]	Trachea	18 (36.7%) [23.2-50.2]
Infectious diseases	8 (16.3%) [6-26.6]	Wound	6 (12.2%) [3-21.4]
Surgery	2 (4.1%) [0-9.7]	Blood	1 (2%) [0-5.9]
Orthopedic	-	Ascites	3 (6.1%) [0-12.8]

ICU: Intensive Care Unit

CI: 95% Confidence interval

Discussion

K. pneumoniae is being increasingly recognized as a clinically significant nosocomial pathogen [1]. Quinolones are among the most commonly administered antimicrobials routinely used for the treatment of serious infections caused by *K. pneumoniae* and other members of the genus Enterobacteriaceae [6]. However, the development of resistance to these antibiotics makes the treatment decision difficult, leading to treatment failures [5]. In recent years, plasmid mediated quinolone resistance among enterobacterial isolates has been reported in several studies, worldwide. However, the number of reports on prevalence of *qnr* genes among Iranian enterobacteria isolates is only limited to few studies [28, 29].

In the present study, 58% and 34.5% of isolates were fully or intermediate resistant to nalidixic acid and ciprofloxacin, respectively. These findings were higher than the two previously conducted studies in Iran. Raei et al demonstrated that 36.2% and 34.1% of urinary *K. pneumoniae* isolates were resistant to ciprofloxacin and nalidixic acid, respectively [30]. In another study from Iran, Zamani et al found that 28.57% and 23.8% of *Klebsiella* spp. were resistant to nalidixic acid and ciprofloxacin, respectively [31]. Hence, the emergence of resistant isolates against broad spectrum antibacterial agents in our hospital settings seems to be linked with improper and widespread administration of these antibiotics.

The present study demonstrates a high prevalence (39.5%) for plasmid-mediated quinolone resistance determinants among quinolone non-susceptible *K. pneumoniae* isolates in Iran. The prevalence rate found in our study is higher than those reported by Kim et al from Korea (10%) [32], Wang et al from China (11.9%) [33], Dahmen et al from Tunisia (16%) [34], Yan et al from China (16.2%) [35], and Wang et al from the United States (11.1%) [36] but still lower than that found by Bouchakour et al in Morocco in which 50% of ESBL-producing *K. pneumoniae* isolates were shown to carry *qnr* determinants [37]. This might be indicative of a rising trend in the rate of plasmid mediated quinolone resistance among the genus of Enterobacteriaceae.

In the current study, 25% of *qnr*-positive isolates were shown to have high level quinolone resistance. As plasmid mediated quinolone resistance determinants produce only low-level resistance to quinolones, it can be hypothesized that high level resistant pattern is possibly

caused by another mechanisms such as chromosomal mutation which was not evaluated in the present study. Considering the findings of the present study, it is obvious that most *qnr*-positive *K. pneumoniae* isolates were mostly obtained from the patients admitted to ICUs. Long term ICU stay, broad spectrum antibiotics intake, chronic underlying conditions, and the application of invasive techniques and devices probably make the patients more susceptible to infections caused by these resistant organisms.

In the present study, 30.6%, 9.7%, and 1.6% of quinolone non-susceptible *K. pneumoniae* isolates carried *qnrB1*, *qnrB4,* and *qnrS1* genes alone or in combination, respectively. We believe that this is the first report of *qnrS1*, *qnrB4,* and *qnrB1* genes among the clinical isolates of *K. pneumoniae* collected from two distinct provinces of Iran. In a study by Pakzad et al reported from Iran, 9 (37.5%) and 4 (20.8%) of ESBL-producing *E. coli* isolates were positive for *qnrA* and *qnrB* genes, respectively [29]. The presence of *qnrA* (25.8%), *qnrB1* (1.17%), and *qnrS* (1.17%) genes among ESBL-producing *Salmonella* spp. was also reported in a study by Saboohi et al from Iran [28]. In another study from Iran, Seyedpour et al showed that 30.4% of community isolates of *K. pneumoniae* harbored *qnr* and/or *aac (6')-Ib-cr* genes [38]. In Taiwan, Wu et al described the presence of *qnrB4* (3.6%), *qnrS1* (2.8, and *qnrB2* (2.3%) genes in the clinical isolates of *K. pneumoniae* [39]. Robicsek et al in the United States reported that 14% and 6% of ceftazidime-resistant *K. pneumoniae* isolates harbored *qnrA* and *qnrB* genes, respectively [40]. Dahmen et al from Tunisia showed *qnrA* was more prevalent among *K. pneumoniae* isolates whereas *qnrB1* was the most prevalent genes among *E. cloacae* isolates followed by *qnrB2* and *qnrS1* [34]. Similarly, Yan et al in their report from China demonstrated that 8.1%, 4.1%, and 4.1% of ESBL-producing *K. pneumoniae* isolates were positive for *qnrA*, *qnrB*, and *qnrS* genes, respectively [35]. Finally, Wang et al in a study carried out in China reported that 62 (15.1%), 25 (6.1%), and 10 (2.4%) of ESBL-producing *K. pneumoniae* isolates were positive for *qnrS, qnrB,* and *qnrA* genes, respectively [33].

Conclusions

Findings of the present study reveal a high prevalence for plasmid-mediated quinolones resistance due to qnr genes among the clinical isolates of *K. pneumoniae* in Iran. The appearance and spread of such resilient organisms within the medical centers around the country not only brings about issues of great concern for human health but also raises questions on how to achieve a successful antibiotic therapy through planning a comprehensive infection control guideline to avoid further spread of these resistant organisms within our medical settings. Our data also highlights the necessity for establishing an appropriate infection control strategy and sensible antibiotic therapy.

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ORIGINAL ARTICLE

Budget impact analysis of universal rotavirus vaccination in the Local Health Unit 11 Empoli, Tuscany, Italy

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Key words

Rotavirus • Vaccination • Budget impact

Summary

Background. *Rotavirus (RV) infection is the first cause of acute viral gastroenteritis in children under five years of age all over the world; it mainly affects children between six and 24 months of age and can cause serious acute diarrhoea and dehydration. The aim of this study is to perform the budget impact analysis of universal rotavirus vaccination in the Local Health Unit (LHU) 11 Empoli, Tuscany, Italy.*

Methods. *An ad hoc mathematical simulation model was developed to evaluate the budget impact analysis of 5-years universal rotavirus vaccination. Particularly, incidence of rotavirus gastroenteritis (RVGE), hospitalizations, nosocomial diarrhoea, medical consultations, prescriptions and accesses to emergency department were taken into account in the analysis. The direct medical costs due to RV diarrhoea and the costs of vaccination campaign were considered as the main outcome measures in the study.*

Results. *The adoption of universal rotavirus vaccination campaign for five years in the LHU 11 Empoli would result in relevant savings due to the health cares avoided. These savings would overlapped the costs of vaccination yet from the second year after the introduction of vaccination. The saving for the Health Service would be 1.5 million Euro after five years of campaign.*

Conclusions. *Universal vaccination against rotavirus results clinically and economically favourable for both the Health Service and the Society perspectives.*

Introduction

Rotavirus (RV) infection is the first cause of acute viral gastroenteritis in children under five years of age all over the world; it mainly affects children between six and 24 months of age and can cause serious acute diarrhoea, resulting in dehydration (which can lead to death if rehydration therapy is not adequately administered) [1-9]. Most of the children infected by RV are under three years of age and many children get sick more than once.

Every year, in the developing countries, rotavirus acute viral gastroenteritis (RVGE) causes the death of about 444,000 children; while in USA these diseases are responsible for a number of hospitalization between 58,000 and 70,000 [10]. In Europe, each year, among the population under five years of age (about 23.6 millions) there are approximately 3.6 millions cases of RVGE, 231 deaths, at least 87,000 hospitalizations and 700,000 medical examinations related to this disease [2].

In industrialized countries, deaths caused by RV are rare because of easy and rapid access to primary cares, but the burden of this disease is very relevant, due to the high frequency of the infection [2].

Several studies, performed in different countries, show the impact of RVGE on primary cares and on hospitalizations, also in the economic perspective; these studies often demonstrate discordant results, probably due to the different study designs applied (methods, populations and aim) [11-21].

In Italy, RV disease has a remarkable clinical impact with repercussions on National Health System (medical examinations, accesses to emergency department, hospitalizations) and on families (absences from work, costs of drugs, dietetic products, diapers, etc.) [22].

As a matter of fact, RV is the main responsible for the hospitalizations due to diarrhoea (about 10,000 hospitalizations per year) in children under five years of age (about 1% in a birth cohort), with an average duration of hospitalization amounting to five days during the last ten years [23-25]. Infection can also be contracted in hospital, causing an increase of the average duration of hospitalization of five days [23, 24].

In Italy, RVGE must be notified in the Second Group of infection diseases (according to Ministerial Decree 15/12/1990) as "infectious diarrhoea, not caused by *Salmonella spp.*", but official surveillance data on incidence of this disease are not available. However, there is a national surveillance system addressed to the characterization of circulating RV strains; this system is part of the European surveillance system [26].

RVGE is an infection preventable by vaccination. Nowadays, two different vaccines against RV are available in Europe: Rotarix (GSK) and Rotateq (Sanofi Pasteur); they must be orally administered, respectively in two and three doses, in children sixth weeks old.
To evaluate the allocative efficiency of this vaccination, considering the limited resources of Health System, and to support the decision makers in management decisions, an economic evaluation on the introduction of universal vaccination against RV for all the newborns in the LHU 11 Empoli, in Tuscany (Italy), for a period of five years, was carried out through a budget impact analysis.

Materials and methods

An *had hoc* mathematical simulation model, in Excel (Microsoft, Redmont, USA), was developed in order to perform the budget impact analysis of the introduction of RV vaccination in the LHU 11 Empoli.
The clinical and economic impact related to the implementation of universal RV vaccination in a five-years period was compared with a no-vaccination scenario.
In the mathematical simulation, a vaccine coverage of 90%, constant for five years, was supposed.
Considering the short horizon time of analysis, no discount rates were applied to costs and benefits. The economic evaluation considered the birth cohorts of the LHU 11 Empoli, that includes about 2,300 newborns per year.
After the literature review, the following parameters were included in the study: incidence of RVGE, hospitalizations, accesses to emergency department, and medical consultations. Particularly, an analysis of the National Health System's direct medical costs associated to the burden of RV disease was carried out. To achieve the budget analysis, not-medical direct costs (transport to the hospital, diapers consumptions, rehydration solutions, drugs, special foods, etc.), and indirect ones (working days lost by the parents due to their sick children) were not considered, because they were both Societal expenses.
The parameters used in the mathematical simulation model are shown in Table I. Particularly, percentages and costs related to RVGE cases, hospitalizations, nosocomial diarrhoea cases, medical consultations, prescriptions and accesses to emergency department, were obtained from two Italian studies concerning economic evaluations [23, 27].

Tab. I. Parameters used in the mathematical simulation model to perform the budget impact analysis.

	Incidence %	Average costs per case (Euro)
RVGE cases	45.45%	-
Hospitalizations due to RV diarrhoea	1.82 %	1,463
Nosocomial diarrhoea cases	0.91%	2,000
Medical consultations	22.73%	23.80
Accesses to emergency department	7.70%	352.72
Prescriptions		9.98

Vaccine data

Data on the efficacy of the RV vaccine used in the mathematical simulation model were obtained from a clinical study performed in several European countries, including Italy [28].
The cost of the RV vaccine in the LHU 11 Empoli, currently of 36.50 Euro for each dose, plus 10% for taxes (price in 2013), was used to develop the economic analysis. No other costs (for example the cost for the further organization of the vaccine centre) was added because the vaccine, already available in co-payment, would have been administered at the same time of the first two vaccination sessions, according to the Tuscan vaccination schedule [29].

Sensitivity analysis

To evaluate the impact of the uncertainty related to the input data on the results, a sensitivity analysis was carried out, applying a variation of ± 20% on the cost of the RV vaccine and on the percentages of hospitalizations, nosocomial diarrhoea cases, medical consultations, and accesses to emergency department. In addition, a variation on the vaccine coverage (from 90% to 80% and 70%) was also applied [23].

Results

Data obtained from the simulation carried out using the mathematical model show that the universal vaccination against RV, in five years in the LHU11 Empoli, would cause a relevant reduction (47%) of the RVGE cases: 26,134 cases of RVGE in no-vaccine scenario and 13,762 cases in vaccine scenario. In Table II, the cases of RV diarrhoea prevented with the introduction of the RV vaccination are shown.

Tab. II. Clinical impact of RV vaccination in LHU 11 Empoli: RVGE cases prevented with the introduction of vaccination, divided by age.

RVGE cases prevented with the vaccination	Children age					
	0 years	1 year	2 years	3 years	4 years	Total
1st year of vaccination	825	0	0	0	0	825
2nd year of vaccination	825	825	0	0	0	1,650
3rd year of vaccination	825	825	825	0	0	2,474
4th year of vaccination	825	825	825	825	0	3,299
5th year of vaccination	825	825	825	825	825	4,124
Total	4,124	3,299	2,474	1,650	825	12,372

Tab. III. Costs for hospitalizations, nosocomial diarrhea cases, medical consultations, prescriptions, accesses to emergency department caused by RVGE and prevented with the vaccination (Euro).

	Hospitalizations for RV diarrhoea	Nosocomial diarrhoea cases	Medical consultations	Prescriptions	Accesses to emergency department	Total
1st year of vaccination	58,791.53	33,027.54	10,426.73	4,372.22	52,347.10	158,965.13
2nd year of vaccination	117,583.07	66,055.08	20,853.47	8,744.44	104,694.21	317,930.26
3rd year of vaccination	176,374.60	99,082.62	31,280.20	13,116.65	157,041.31	476,895.39
4th year of vaccination	235,166.13	132,110.16	41,706.93	17,488.87	209,388.42	635,860.51
5th year of vaccination	293,957.66	165,137.70	52,133.66	21,861.09	261,735.52	794,825.64
Totale	881,872.99	495,413.10	156,400.99	65,583.27	785,206.57	2,384,476.93

Costs for hospitalizations, nosocomial diarrhoea cases, medical consultations, prescriptions and accesses to emergency department prevented with the RV vaccination are shown in Table III. The LHU 11 Empoli could save about 882,000 Euro for hospitalizations, 495,000 Euro for nosocomial diarrhoea cases, 156,000 Euro for medical consultations, 66,000 Euro for prescriptions and 785,000 Euro for the accesses to emergency department in five-years vaccination period.

Vaccination costs

Vaccination against RV for a birth cohort (2,300 newborns per year) in the LHU 11 Empoli would amount 166,000 Euro per year, with a total cost of 831,000 Euro after five years from the introduction of the vaccine in the schedule.

Budget impact analysis

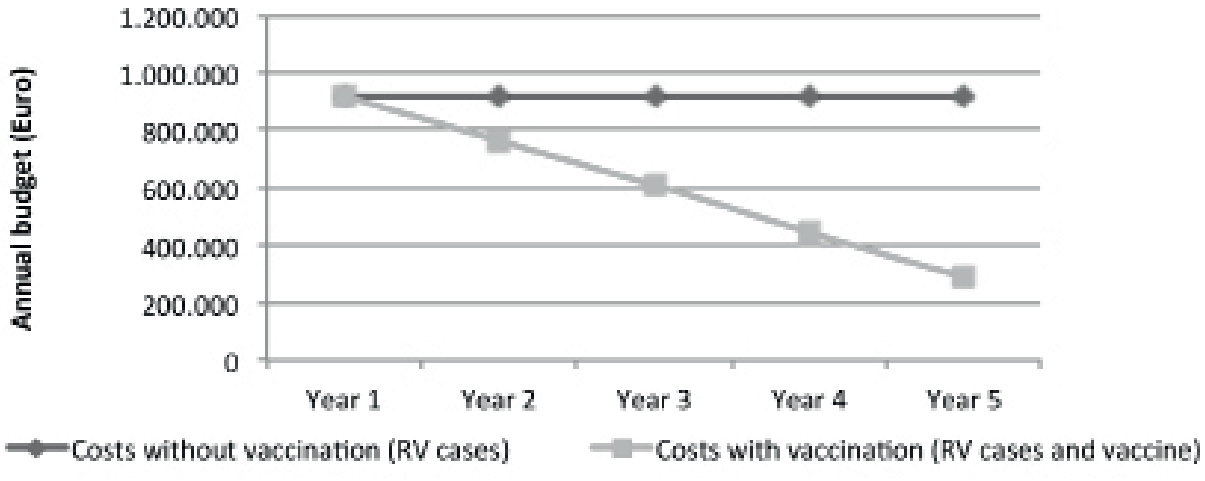
According to the mathematical method, the annual cost related to the RV disease for the LHU 11 Empoli without the adoption of an immunization program would be about 916,000 Euro (Tab. IV).

During the first year of RV vaccination, the costs for the Health Unit to take care of the remaining cases of RV disease, added to the costs related to vaccination, would exceed the costs related to the disease burden; from the second year, savings are registered and increase in the following years as shown in Figure 1. The total savings

Tab. IV. RV disease costs with and without RV vaccination and budget difference (Euro).

Budget difference (Euro)	Year 1	Year 2	Year 3	Year 4	Year 5
RV disease costs without vaccination	916,139	916,139	916,139	916,139	916,139
Costs for remaining cases of RV disease with vaccination	757,174	598,208	439,243	280,278	121,313
Vaccination cost	166,221	166,221	166,221	166,221	166,221
Budget difference	-7,256	151,709	310,674	469,640	628,605
Total budget difference in 5 years-period	1,553,372				

Fig. 1. Costs of RV disease without RV vaccination compared with disease costs after the introduction of RV vaccination (as sum of RV disease costs and vaccination campaign costs).



Tab. V. Total costs and saving in the five-years period: sensitivity analysis.

	RV disease costs without vaccination (Euro)	Costs due to remaining cases of RV disease with vaccination (Euro)	Budget difference (Euro)
Model	4,580,693	3,027,322	1,553,372
Vaccination coverage 80%	4,580,693	2,934,977	1,645,717
Vaccination coverage 70%	4,580,693	2,842,632	1,738,062
% Hospitalizations: -20%	4,311,501	2,913,184	1,398,317
% Hospitalizations: +20%	4,816,236	3,127,192	1,689,045
% Nosocomial diarrhoea: -20%	4,339,193	2,900,148	1,439,046
% Nosocomial diarrhoea: +20%	4,799,193	3,142,384	1,656,810
% Medical consultations: -20%	4,492,705	2,983,574	1,509,131
% Medical consultations: +20%	4,651,978	3,062,764	1,589,214
% Accesses to emergency department: -20%	4,276,472	2,876,063	1,400,410
% Accesses to emergency department: +20%	4,824,070	3,148,328	1,675,742
Vaccine cost: -20%	4,580,693	2,860,687	1,720,007
Vaccine cost: +20%	4,580,693	3,159,802	1,420,892

for the LHU 11 Empoli would be of 1,553,372 Euro at the end of the five years.
As shown in Figure 1, the savings due to the adoption of the RV vaccination would be registered after the second year of immunization program.
Also changing the input data in the mathematical method, the adoption of the universal RV vaccination program for the newborns in the LHU 11 Empoli is still economically favourable for the payer (LHU) (Tab. V).

Discussion and conclusions

Nowadays, also in Italy, infections caused by Rotavirus in children from zero to five years of age results in an high clinical and economic impact, in spite of the availability of effective vaccines.
The results of the analysis carried out show that the adoption of an universal vaccination programme against RV, in five years in the LHU 11 Empoli, Tuscany (Italy), would determine considerable savings related to the treatments and therapies avoided with the implementation of immunization campaign. These savings would exceed costs for vaccination since the second year of the vaccine programme. Therefore, also in more limited time horizon of analysis, for example 3 years, typical of budget impact evaluations, our results continued to be favourable with saving amounting to 455,128 Euro.30 As a matter of fact, the RV vaccination cost represents the 0.5% of the annul pharmaceutic budget in the LHU 11 Empoli, without considering the obtained saving.
The results of the study would be more favourable, from the point of view of the Society, considering also not-medical direct costs and indirect costs avoided with the introduction of immunization.
Data obtained agree with other studies carried out in European countries, for example in France, but disagree with analysis performed in England and in the Netherlands, where vaccination against RV is not cost-effective in the current situation, and with the studies concerning United States, where RV vaccination would be cost-effective but it would not determine savings for the payer [10, 21, 30-32]. In spite of this, the RV universal vaccination for newborns was recently implemented in England.
In Italy, two studies demonstrated that the introduction of RV vaccination was favourable, particularly in terms of cost-effectiveness, at national and regional level [23, 27]. In addition, our results are consistent with data obtained in two other studies on RV vaccination conducted in the Province of Genoa (Northern Italy), and should be considered conservative if the RV hospitalisation code is attributed only to 65% of RV-positive cases and, consequently, hospitalisation related to RV is underestimated, as showed in that analyses [33, 34].
In this study, a vaccination coverage of 90% was supposed in order to evaluate the greatest impact of the RV vaccination. This assumption implies the underlying rationale that the co-administration of RV vaccine with hexavalent vaccine would determine the rapid achievement of high level of vaccination coverage.
However, the sensitivity analysis demonstrates that, also reducing the vaccination coverage to 70%, as in other studies reported in literature, the favourable economic issue of rotavirus vaccination still stand [31, 32].
In this study, the effect of herd immunity was not taken into account: according to some authors, it would increase the overall effectiveness of RV vaccination [32, 34, 37].
The study has some limitations: epidemiological data and costs were collected from a recent study and they were not directly calculated in our area, due to a gap of specific data [27].
However, data used in the mathematical simulation were consistent with local data obtained from health archives, considering that the underestimation about RV diarrhoea cases and hospitalizations is about 40% [24], due to the high number of cases of gastroenteritis with not-defined aetiology, related to the lack of sensitivity of the hospital discharge data system[20, 31, 32, 35, 38].
Indeed, the examination of the hospital discharge data of the period between 2004 and 2013 [36] 2013 [39] in the LHU 11 Empoli, shows that 337 children between zero and five years of age were hospitalized because of RV

disease or they extended the period of hospitalization due to RV. They determine a total cost exceeding 500,000 Euro, assuming real costs of hospitalization equal to Diagnosis-Related Groups (DRG) [37, 40] charges (even if it was demonstrated that these costs often exceeded the applied charges, especially in paediatric wards) [35, 38]. These hospitalizations concerned only diarrhoea cases in which a clear RV aetiology could be proved and these data are consistent with those reported in the study [1-3]. In this analysis, the costs for National Health System and costs for the families, that might result from possible adverse drug reactions to vaccination, were not taken into account, because of the rarity of these events. In addition, costs for possible paediatric consultations at home were not considered in the mathematical simulation.
The budget impact analysis carried out demonstrates that the adoption of RV universal vaccination for the newborns cohorts in 5 years would be clinically and economically favourable for both the Health Service and, consequently, the Society.

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ORIGINAL ARTICLE

Effect of mass media on influenza vaccine coverage in the season 2014/2015: a regional survey in Lazio, Italy

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Key words

Mass media • Vaccine coverage • Anti-flu vaccine

Summary

Introduction. *Adherence to vaccination program for Influenza virus is an important issue of Public Health in presence of many no-vaccine tendencies. The media event about some deaths, occurring after MF59 adjuvanted vaccine administration, has characterized the season 2014/15 vaccination program in Italy. Aim of the study is vaccination adherence assessment of the current season with regards to local health units (LHU) coordinators's perceptions in Lazio Region (IT).*
Methods. *LHU coordinators's perceptions were collected from a questionnaire that was send via email to the all 12 LHU coordinators. The questionnaire was built with 4 questions concerning the impression about the vaccination adherence of elderly people in the current season. Data from questionnaire was compared with the official coverage rate obtained by the Regional Authority. Severe adverse events were collected by 1 LHU.*
Results. *All the 12 LHU coordinators answered to our questionnaire: 7/12 (50%) predicted a coverage rate of at least 50%; 3/12 (25%) referred a coverage rate around 40-45%; 2/12 (17%) predicted a reduction of 5-10% less than the previous season. Indeed, a mean 49.1% vaccination coverage in the elderly has been reported by the Regional Authority highlighting a reduction of 10% less than the 2013/14 season coverage. No severe adverse events were observed.*
Discussion. *In our survey an important effect of media event on anti-flu vaccination program adherence has been evidenced, with a failure in communication and joint management of Public Health Institutions in Italy about efficacy and safety information of flu vaccine.*

Introduction

Influenza viruses are the etiological agents for flu illness, an important cause of morbidity and mortality worldwide. Flu easily spreads through the population and is present year round in the tropics or during winter in the temperate regions. Influenza affects globally 5-10% of adults and 20-30% of children each year. Cases of severe illness are estimated about 3 to 5 millions, with a mortality around 250 to 500 thousands [1-4]. In Europe about 10% of the population presents symptoms of flu with hundred million hospitalizations yearly [5]. In Italy, flu illness affects an average 8% of the population [6] and causes more than 8,000 deaths per year due to fatal consequences [7]. The clinical manifestations of the disease are not typical, and the term Influenza-Like-Illness (ILI) is used, which includes acute self-limited febrile respiratory symptoms with muscular pain, asthenia, headache and malaise [8]. Case definition of ILI has been recently updated from European Centre for Disease Prevention and Control (ECDC), and it is defined as the sudden onset of fever, malaise, headache or myalgia, associated with at least one of respiratory symptoms such as cough, sore throat or shortness of breath [9]. Course of illness is generally mild; however, in some groups (elderly people, infants, pregnant women and patients with chronic diseases such as diabetes, cardiovascular disorders, asthma, BPCO or immunodeficiency) important consequences can be experienced. These complications include: primary influenza viral pneumonia [10], chronic illness exacerbation, secondary bacterial pneumonia, sinusitis, otitis media, coinfections with other viral or bacterial pathogens [7, 11, 12].

To prevent these complications an adequate vaccination program is important to be planned. The importance of preventing influenza is mainly for the abovementioned groups with an increased risk to develop flu complications. In particular, vaccination is important in the elderly (people with an age ≥ 65 years), as they present a reduced immunological response to microbial agents [13, 14]. As influenza viruses are highly mutant, vaccine is built from the new circulating viral components; consequently, annually vaccine formulation made by a global surveillance coordinated by World Health Organization (WHO) is required [15]. Once vaccine is produced, Public Health Institutions coordinate each year vaccine campaigns for influenza prevention. In Italy, official recommendation for flu prevention program is published each year by Ministero della Salute (MdS). Recommendation indicates risk groups to whom vaccine has to be offered and the formulations

appropriate for each age groups. Vaccine campaign is performed from October to December [16].
In Italy during 2014/15 flu season, vaccination campaign has been characterized by an important media event, which negatively influenced the coverage. Four deaths occurred from 1 hour to 5 days after MF59 adjuvanted vaccine administration in the period 7-24 November 2014. Then in November 27, two batches of the MF59 adjuvanted vaccine were precautionarily withdrawn by the Agenzia Italiana del Farmaco (AIFA), and exhaustive analyses about their safety were performed [17]. Lazio Region decided to suspend the distribution of all batches of MF59 adjuvanted vaccine, after 2 additional suspected deaths were reported in the Region [18, 19].
In December 3, the European Medicine Agency (EMA) established the absence of relationship between deaths and vaccine administration [20]. To this date, an overall 19 deaths after MF59 adjuvanted vaccine administration were reported to AIFA. In December 24, after Istituto Superiore della Sanità (ISS) had verified vaccine safety with laboratory tests [21], AIFA removed the block of the two batches [22]. After obtaining the positive advice of MdS, Lazio Region extended flu vaccination campaign up to January 31, 2015 [23].
In this situation, the emphasis of mass media on reporting these news determined an increasing fear about flu vaccine and vaccination that spread through the Italian population.
Aim of the study is to assess the vaccination adherence following the media event in more than 1,200,000 people aged ≥ 65 years, resident in Lazio Region, Italy.

Methods

A questionnaire was built with the following four topics: flu vaccine distribution, adherence at campaign startup, media event effects, coverage projection in ≥ 65 years population. In January 26, 2015, the questionnaires were sent via email to coordinators of the influenza campaign or vaccinations for the 12 local health units (LHU) in Lazio. Results of questionnaire were collect via email or telephone from January 27 to February 17. Moreover, data of severe adverse events after vaccine administration were collected from one LHU.
Adverse events were investigated in subjects aged ≥ 65 years immunized with MF59 adjuvanted vaccine, through a 7-days monitoring (namely active surveillance) or through a questionnaire sent to all general practitioners in that LHU (i.e. passive surveillance).
The distribution of available vaccines in the different LHU and the 2013/14 and 2014/15 vaccine coverages at the end of November and at the end of the campaign were obtained from the Regional Authority.
Descriptive analyses were made based on the answers to 4 questions. The results of these analyses were subsequently compared with data of vaccine coverage.

Results

The responses to the questionnaire were collected from 12/12 coordinators of LHU. MF59 adjuvanted vaccine was available in 12/12 LHU, 10/12 (83%) coordinators specified the use in ≥ 65 years people for whom the vaccine is licensed.
One or both suspected batches were present in 7/12 (58%) LHU.
Almost all the LHU coordinators (11/12) reported a startup campaign better or similar to that of the previous year; only 1/12 (8%) referred a low initial adherence to vaccination program (Fig. 1).
A personal opinion was asked about the impact of the media events on the vaccination campaign as Figure 2.

Fig. 1. LHU coordinators' perception on flu vaccination campaign startup adherence.

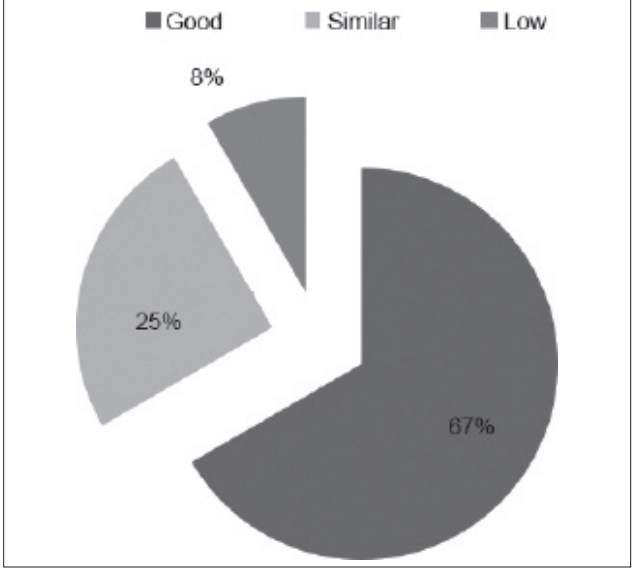
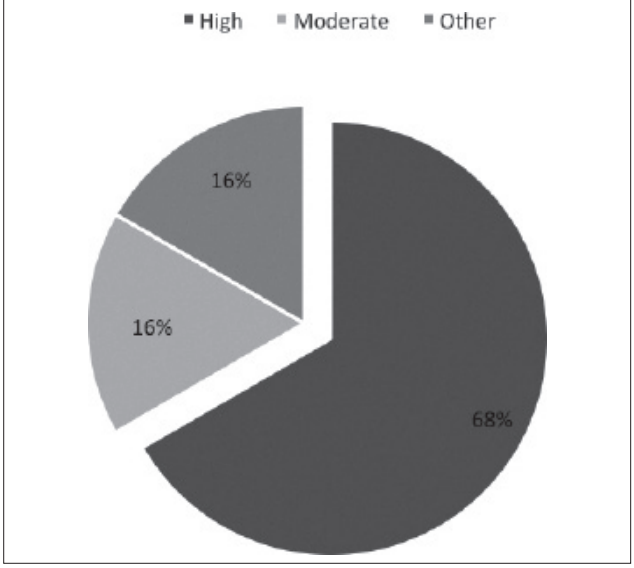


Fig. 2. LHU coordinators' judgement about media event impact on the 2014/15 flu vaccination campaign.



Tab. I. Coverage rate of vaccination among elderly in flu season 2013/14 and 2014/15.

LHU	Elderly resident in Lazio 2013/14*	Influenza Season 2013/14		Estimated LHU Projections	Elderly resident in Lazio 2014/15**	Influenza Season 2014/15	
		Vaccinated	Coverage Rate (%)	Coverage Rate (%)		Vaccinated	Coverage Rate (%)
LHU 1	124163	55207	44.5	45.0	127033	47529	37.4
LHU 2	139543	76522	54.8	50.0	142323	70863	49.8
LHU 3	133991	67743	50.6	46.0	136907	62710	45.8
LHU 4	123031	67609	55.0	50.0	125495	63572	50.7
LHU 5	122856	56880	46.3	46.0	125504	51530	41.1
LHU 6	56564	32354	57.2	45.0	57692	29740	51.5
LHU 7	86500	51279	59.3	50.0	88251	47223	53.5
LHU 8	97343	52874	54.3	53.0	99273	49882	50.2
LHU 9	71759	41299	57.6	50.0	73292	37873	51.7
LHU 10	38539	21904	56.8	55.0	39381	18385	46.7
LHU 11	103017	66567	64.6	59.0	105104	62740	59.7
LHU 12	104187	63357	60.8	50.0	106431	60586	56.9
TOT	1201495	653595	54.4	49.7[a]	1226686	602633	49.1

LHU: Local Health Unit

* Data from ISTAT 01/01/2014

** Data from ISTAT 01/01/2015, central hypothesis

[a] Adjusted by ISTAT 01/01/2014

In particular: a very negative impact of media event was generally perceived by 8/12 (68%) LHU, sometimes describing a real refusal of flu vaccination with subsequent important adherence lowering; 2/12 (16%) specified a moderate reduction of the vaccination adherence; 2/12 (16%) coordinators quantified an hypothetical percentage of adherence reduction around 10-15%.

The LHU coordinators gave their personal projections regards to vaccination coverage in the ≥ 65 years population: 7/12 (58%) predicted at least 50% coverage; 3/12 (25%) estimated a coverage less than 50%, included in a 40-45% range; 2/12 (17%) answered in terms of coverage reduction, reporting a 5-10% decrease compared with the previous influenza vaccine campaign.

Using these data, a weighted mean 49.7% vaccination coverage in people ≥ 65 years was expected, with an estimated reduction around 10% compared to the previous seasonal coverage data (54.4%), shown in Table I.

Coverage data from Regional Authority showed a mean rate among the 12 LHU of 54.4% and 49.1% for season 2013/14 and 2014/15 respectively (Tab. I), with a decrease in all LHU ranging from 6 to 18%. In particular, 3/7 (43%) coordinators of LHU with the suspected batches gave an overestimation of effective coverage in elderly, 4/7 (57%) gave the same or a quite lower estimation than the real coverage data. Despite that, 4/5 (80%) of the LHU without the suspected batches gave an estimate of reduced coverage caused by the media impact similar to the value reported by the Regional Authority. Interestingly, 4/12 (33%) coordinators had overestimated as well as 4/12 (33%) had underestimated the coverage reduction, without any correlation with the presence of the suspected batches.

Moreover, a reduced adherence (around 7%) in the first 2 months of 2014/15 season for elderly has been evidenced when compared with the previous seasonal adherence at startup.

No evidence of severe adverse events were identified from both active and passive surveillances. Active surveillance was performed in 95 subjects, of whom 68/95 received the suspected batches of MF59 adjuvanted vaccine. Among general practitioners, 97/456 answered to the questionnaire reporting 9,483 subjects immunized with MF59 adjuvanted vaccines, of whom 5,916 subjects received the batches under investigation.

Discussion

Vaccination adherence is an important issue about which Public Health Institutions debate for several years. Vaccination programs have surely reduced in the last years the burden of many infectious diseases with regard to incidence, morbidity and mortality. As a consequence of this success, general population looks mostly at the adverse events to vaccines and underrate their beneficial preventive effects. Due to a supposed low utility, a disputable feeling for vaccine is recently organized, enhanced and conducted into several no-vaccine tendencies, which strengthen the fear about vaccine utilization also with publishing no scientific papers [24]. In particular, one of the main object of the debate for these no-vaccine tendencies is anti-flu vaccine, caused by its annual program planning, production and distribution. Moreover, general population don't think that anti-flu vaccine is necessary, as influenza is a disease with a good prognosis.

These considerations cause a low amount of people to which vaccine is annually administered, that is often lesser than the minimal coverage target built on a 75% of people ≥ 65 years by Public Health Institutions [25]. This point is well evidenced in Italy, where a mean flu-vaccine coverage around 60% (range 54.2-68.3%) is observed in the last 10 years. In particular, in Lazio Region a progressive low decline in anti-influenza vaccine coverage has been observed in the last years, after a peak of 74.1% registered in 2006/07 [26]. In 2013/14 season the declining trend seemed to get a stop and a lot of work had been planned to increase coverage.

In the matter of question, season 2014/15 was characterized by an important reduction in terms of adherence to vaccination for elderly people, caused by the obsessive fear after the media event of suspected deaths related to MF59 adjuvanted vaccine administration. The perception of LHU coordinators is that this fear for flu-vaccine was induced and not well managed by the different Italian Public Health Institutions, as they did not take in consideration the media impact for deaths, did not pay the right attention to better transmit the vaccine safety to general population and, most important, they did not act univocally.

The media event occurred in Italy highlighted the problem of adverse events management following immunization. According to WHO recommendations, serious adverse events (e.g. deaths) have to be evaluated in order to determine the causal or only temporal association [27].

In this survey, the LHU coordinator's perception in Lazio Region has been that the vaccine coverage in elderly was about 10% less than 2013/14 season, although they felt campaign starting better than the previous one. This perception has been already confirmed for the reduced low adherence of elderly decreasing from 54.4% of the previous season to 49.7% of 2014/15 season in all LHU, without a correlation between perception and real decrease rate. This lack of correlation could be explained by the fact that around 99% of people ≥ 65 years resident in Lazio has been vaccinated by the general practitioners (GP) and not by physician of LHU, with a GP/LHU ratio of 122:1. Therefore, LHU coordinators did not receive a real-time information about vaccination coverage in the elderly.

Moreover, we have pointed out that the media event has determined an important flu vaccination coverage reduction in the LHU using the suspected batches. The decrease was, however, detected also in the LHU not having these batches, in those using only a small amount of MF59 adjuvanted vaccine and even where both active and passive surveillance did not recognize any severe adverse event.

However, this low adherence was already observed at startup of vaccination program, because a coverage of about 7-9% less than the previous season is reported from official data by Regional Authority. In fact, a 42.5% mean coverage in elderly has been reported at the end of November 2014 in respect to the 46.6% reported in November 2013.

These considerations highlight an extreme precaution for Italian Public Health Institutions in dealing the effects of media event [28], in particular for Lazio Region with the suspension of all MF59 adjuvanted vaccine batches. In fact, after the vaccine was available again the Region proposed to prolong the campaign up to the end of January; however, there was a delayed final approval from MdS and some LHU coordinators referred a late official communication press, which took place after several general practitioners had already given back to LHU all vaccines.

Conclusions

The media event about the suspected deaths related to MF59 adjuvanted vaccine administration has highlighted a failure in communication and cooperation of Public Health Institutions in Italy. A good management of vaccine planning, as well as improving health workers knowledge for vaccine safety and effectiveness, are needed to ensure the right level of vaccination coverage and increase the acceptance of immunization by the target population.

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ORIGINAL ARTICLE

Conley Scale: assessment of a fall risk prevention tool in a General Hospital

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Key words

Conley Scale • Fall prevention • Clinical risk management

Summary

Introduction. *"Umberto I" Teaching Hospital adopted 'Conley scale' as internal procedure for fall risk assessment, with the aim of strengthening surveillance and improving prevention and management of impatient falls.*
Materials and methods. *Case-control study was performed. Fall events from 1st March 2012 to 30th September 2013 were considered. Cases have been matched for gender, department and period of hospitalization with two or three controls when it is possible. A table including intrinsic and extrinsic 'fall risk' factors, not foreseen by Conley Scale, and setted up after a literature overview was built. Univariate analysis and conditional logistic regression model have been performed.*
Results. *50 cases and 102 controls were included. Adverse event 'fall' were associated with filled Conley scale at the admission to care unit (OR = 4.92, 95%CI = 2.34-10.37). Univariate analysis identified intrinsic factors increasing risk of falls: dizziness (OR = 3.22; 95%CI = 1.34-7.75), psychomotor agitation (OR = 2.61; 95%CI = 1.06-6.43); and use of means of restraint (OR = 5.05 95%CI = 1.77-14.43). Conditional logistic regression model revealed a significant association with the following variables: use of instruments of restraint (HR = 5.54, 95%CI = 1.2-23.80), dizziness (OR = 3.97, 95%CI = 1.22-12.89).*
Discussion. *Conley Scale must be filled at the access of patient to care unit. There were no significant differences between cases and controls with regard to risk factors provided by Conley, except for the use of means of restraint. Empowerment strategies for Conley compilation are needed.*

Introduction

Patient falls are one of the most common adverse events and one of the most important issue in the context of risk management, both for the frequency with which they occur, and for the consequences that may ensue. Some studies show that falls represent up to 70% of hospital accidents, entailing a direct physical and psychological impact [1, 2]. Hospital falls, that involve death or serious harm for the patient, are treated, in Italy, as a *sentinel event* by Ministerial Protocol [3].
Falls have a multifactorial etiology and more than 400 risk factors have been described.
The incidence of this phenomenon is prevalent in the elderly population, but the event can also occur in younger subjects; the causes may be attributable to diseases or physiological situations, fall is an event that, for frequency and consequences entailed, requires a multidisciplinary approach, articulated at different levels, aimed at the prevention and containment of risk, especially among older people [4].
Some studies [5, 6] classify falls as:

- *predictable*: they occur in individuals exposed to identifiable risk factors (disorientation, difficulty in walking, gait changes caused by neurological diseases, etc.);
- *unpredictable:* determined by physiological conditions unpredictable until the fall, falls that cannot be predicted 'a priori' (presence of seizures, dizziness, drug reactions, syncope);
- *accidental:* falls attributable to environmental factors or fatality: when the person falls unintentionally (eg slipping on the wet floor).

14% of falls in hospital are classified as accidental, 8% as unpredictable, while the remaining 78% are included between predictable falls.
Risk of falling cannot be completely eliminated by care settings, but the early identification of patients at higher risk, allows us to identify and focus prevention, on a population that really needs [7]. In order to reduce the incidence of this adverse event, some risk assessment instruments are used to identify patients at risk.
According to WHO, risk factors can be divided in two categories:

a. *intrinsic factors,* which concern to patient health condition, they include data on reason of health care admission, co-morbidities and drug therapies;
b. *extrinsic factors*, related to the organizational aspects of the hospital structure, environmental features and ergonomic resources, or health facilities employed [8].

Outcomes related to the adverse event 'fall' are a cause of costs increasing, due to prolonged hospitalization and further diagnostic and therapeutic procedures needed [9]. Moreover, in terms of quality of care, this kind of event can take on a negative ethical and legal features, for operators involved.
The rating scales for risk of falls can be used both at the entrance of the patient in the hospital and for subsequent

monitoring during hospitalization. Different scales have been constructed and applied during the last 20 years, eg. Conley, Morse, Tinetti, Stratify [10-17].
However, a critical review of the literature published in 2003 emphasizes that none of these scales seems to have been sufficiently studied in terms of validity and reliability [18]. A problem of particular importance is the poor reproducibility on populations different from the experimental ones, that can result in an excessive expenditure of resources in front of a little benefit in terms of prevention [18]. Examining the different risk factors included in the risk assessment scales, it is easy to argue that some items are detected in two or more of them, even if with different formulations. Moreover, the most recent critical review of literature made possible to select and confirm a set of risk factors most important in terms of predictability, in particular: history of falls, balance and gait problems, impaired mobility, vision, orthostatic hypotension, use of different drugs, psychomotor status [19]. Latest trends consider risk assessment not based on the use of items and scores 'preformed', but rather on a detected *core* of individual variables.
Conley Scale [10] was developed by D. Conley in the United States during a study conducted within medical and surgical Units, in 1999. An article regarding Conley scale, has established values of sensitivity around 69%, and specificity between 41% and 61%, therefore, the use of this clinical tool is justified by the good margin of accuracy and predictability in identifying true positives patients truly exposed to the risk of fall [20].
The main objective of this study was to identify factors that can significantly influence the phenomenon 'patient falls' in the hospital, and to describe conditions in which the event 'fall' occurs through monitoring application of Conley Scale, in different hospital departments. A case - control study was performed in the Teaching Hospital "Umberto I" in Rome.

Methods

Sample detection

In the present study cases have been defined as "patients who have had an accidental fall during their hospitalization", from March 1st 2012 to September 30th 2013. Setting of research was Umberto I General Hospital in Rome. Data were collected through the analysis of medical records which contains, among other information, the "SHEET OF FALL DESCRIPTION"; sent to the Risk Manager of the Hospital at the moment of adverse event occurred.
Cases have been excluded from analysis for the following reasons:

- "SHEET OF FALL DESCRIPTION" not properly filled in, so that was not possible to trace the number SDO (Hospital Discharge Data) of the medical record (N = 2);
- regime of hospitalizations different from the *ordinary* one.

Moreover the cases included were paired with two (N = 48 cases) or three controls (N = 2 cases).
The selection criteria for controls were:

- ordinary regime of hospitalization;
- hospitalization during the day of the event fall of case;
- no falls during hospitalization;
- hospitalization in the same care unit of the respective case;
- pairing by gender.

Exclusion criteria for controls was:

- discharge before case fall.

The computerized system GIPSE (Management Information First Aid and Emergency) has been used for detection of controls, it is a technical tool for the computerization of Emergency Services, and disclosures discharge required by SIES (Emergency Medical Information System).
The search criteria considered to determine the controls have been the department, the event date fall (admissions have been considered starting from the four days before case fall) and gender. Medical folders have been identified by SDO codes that stemmed entering this information into GIPSE program.

Description of measuring tool

A table built ad hoc has been used to extract data (Appendix 1). The framework has been divided into three sections: the first two had to being filled in for cases and controls, and the third part was dedicated to cases data collection.
The first section refers to socio-demographic data, related to hospitalization and conditions of the patient at admission: date of birth, age, gender, residence, nationality, SDO code of medical records, admission date, discharge date, department of hospitalization, type (ordinary, day hospital), type of admission (emergency, programmed, transferred), principal diagnosis and secondary diagnoses coded ICD9-CM possible critical condition / instability of the patient at the time of admission and the four risk factors of WHO (Patient ≥ 65 years; taking > 4 drugs; weakness in the hips; unstable equilibrium).
The second section examines some of the information contained in the medical record relatively: data of admission day, presence or not of fall, filled or not filled Conley scale (at admission and any subsequent updates: the scale should be completed within 24 hours of admission or, after clinical stabilization if the admission occurred in emergency departments). Subsequent assessment should be repeated: whenever a change is detected in patient's clinical condition, eg. after surgery, changes in therapeutic, addition or replacement of sleep-inducing drugs, anti-anxiety drugs, psychotropic drugs, benzodiazepines, diuretics etc.; after 72 hours from the first assessment; every 5 days after the second evaluation until discharge; after a fall, at the time of discharge / transfer [20]); bodily functions on the date of case fall; intrinsic and extrinsic risk factors present at time of case fall.
The third section reports the same data present in the fall report form.

Data collection

Hospital records have been formally requested through a letter submitted to the Health Management Department of Teaching Hospital Umberto I.
Folders have been divided between cases and controls and controls hospitalization period has been assessed to include the day of the respective fall case.

Each folder has been analyzed independently by two reviewers using the data extracting form preformed. Medical records evaluations were compared and any disagreement has been resolved with the help of a third reviewer. Finally, data collected by extraction form have been inserted in an Excel file.

In the statistical analysis, possible associations between patient falls and variables have been evaluated using Chi-square test, Fisher's exact test, Student's t, Mann-Whitney and odds ratio (OR), with confidence intervals (CI) at 95%. The level of significance for all analyzes has been setted at $p < 0.05$.

A conditional logistic regression was used to investigate the relationship between the outcome of being a case or a control and the set of prognostic factors (intrinsic and estrinsic). Parameters were estimated using conditional method for matched data [21]. The model fitted only included covariates resulted significant at univariate analysis. The likelihood-ratio test has been applied to evaluate the goodness of model. Data were analyzed using SPSS 19.0.

RESULTS

Descriptive analysis of the sample

50 cases and 102 controls have been identified (Tab. I). The 71.7% of the sample were male, 62.3% was 64 years old and 91.3% had Italian nationality.

66% (33/50) of fall cases occurred in Neurology and Psychiatry Department, 8% (4/50) in Infectious Diseases Department and 4% (2/50) in Orthopedics Units, 4% (2/50) in Emergency Department.

With regard to Conley scale criteria, established by WHO, the present data confirmed that: the average age of cases was 69 years, while 64 years among controls. The difference was statistically significant ($p = 0.001$). 54% of the sample took more than 4 drugs, 31% had critical clinical conditions at the admission, 37.8% showed hips weakness and 45.6% had unstable balance. 46% of hospitalizations featured a filled Conley scale at the admission to care unit. In addition to the main diagnosis, comorbidities informations have been selected among anamnesis data, and thereafter collected and compared between cases and controls. The most part of cases presented one comorbidity

Tab. I. Description of sample.

Variables		N (%)	Missing
Gender	M	109 (71.7)	0
	F	43 (28.3)	
Nationality	Foreign	13 (8.7)	0
	Italian	137 (91.3)	
Age > 64 years	Yes	94 (62.3)	1
	No	57 (37.7)	
The number of drugs taken > 4	Yes	79 (54)	1
	No	67 (45.9)	
Critical conditions	Yes	47 (31)	1
	No	104 (68.9)	
Hips weakness	Yes	56 (37.8)	4
	No	92 (62.2)	
Unstable balance	Yes	67 (45.6)	5
	No	80 (54.4)	
Conley filled in at the admission into Care Unit	Yes	69 (46)	2
	No	81 (54)	

Tab. II a. Univariate analysis between cases and controls.

Variables		Controls (not fallen) N (%)	Cases (fallen) N (%)	P	OR (95%CI)
Gender	M	75 (73.5)	34 (68.0)	0.48[a]	1.3 (0.62 – 2.73)
	F	27 (26.5)	16 (32.0)		
Nation	Italian	91 (91.0)	46 (92.0)	0.99[b]	1.13 (0.33 – 3.9)
	Foreign	9 (9.0)	4 (8.0)		
Pt > 64 years	Yes	58 (57.4)	36 (72.0)	0.08[a]	1.91 (0.92 – 3.96)
	No	43 (42.6)	14 (28.0)		
Pt > 4 drugs	Yes	53 (54.6)	26 (53.1)	0.86[a]	0.94 (0.47 – 1.87)
	No	44 (45.4)	23 (46.9)		
Critical clinical conditions	Yes	27 (26.7)	40 (40.0)	0.1[a]	1.83 (0.89 – 3.74)
	No	74 (73.3)	30 (60.0)		
Hips weakness	Yes	37 (37.4)	19 (38.8)	0.86[a]	1.06 (0.52 - 2.15)
	No	62 (62.6)	30 (61.2)		
Unstable balance	Yes	42 (42.9)	25 (51.0)	0.35[a]	1.39 (0.70 - 2.76)
	No	56 (57.1)	24 (49.0)		
Conley filled at admission	Yes	42 (42.9)	25 (51.0)	< 0.001	4.92 (2.34 – 10.37)
	No	56 (57.1)	24 (49.0)		
		Average (SD)	Average (SD)	p	95%CI
Age		63 (21)	69 (12)	0.53[c]	(-10.89- 0.065)
		Average (SD)/ median (min;max)	Average (SD)/ median (min;max)	p	95%CI
Conley Score		2,9 (± 1.97) / 3 (0;5)	3,27 (± 2.66) / 2 (0;9)	0.96[d]	(-1.89- 1.17)

pt : patient

[a] p-value chi-square[2] test

[b] p-value Fisher exact test

[c] p-value t-student test for independent samples with unequal variances

[d] p-value Mann-Whitney Test

Tab. II b. Univariate analysis between cases and controls.

Variables		Controls (not fallen) N (%)	Cases (fallen) N (%)	P	OR (95%CI)
Hypotension: Systolic BP ≤ 90	Yes	2	2	0.61[b]	1.95 (0.26 – 14.34)
	No	90	46		
Hypertension: Systolic BP ≥ 180	Yes	2	2	0.61[b]	1.93 (0.26 – 14.18)
	No	89	46		
Fever≥ 38°	Yes	4	2	0.99[b]	1.08 (0.19 – 6.15)
	No	91	42		
Not integrity of consciousness state	Yes	7	3	0.99[b]	0.80 (0.19 – 3.29)
	No	77	41		
Disoriented	Yes	13	8	0.58[a]	1.30 (0.50 – 3.39)
	No	89	42		
Dizziness	Yes	11	14	**0.007[a]**	3.22 (1.34 – 7.75)
	No	91	36		
Impaired driving, creeping step, broad base of support, unstable march	Yes	45	25	0.494[a]	1.27 (0.64- 2.50)
	No	57	25		
Agitated	Yes	11	12	0.033[a]	2.61 (1.06 – 6.43)
	No	91	38		
Impairment of judgment / lack of the sense of danger	Yes	12	12	0.05[a]	2.37 (0.98 – 5.74)
	No	90	38		
Reduced muscle strength	Yes	45	20	0.59[a]	0.83 (0.42 – 1.65)
	No	56	30		
Patient with impaired vision	Yes	13	5	0.62[a]	0.76 (0.25 – 2.27)
	No	89	45		
Incontinence (fecal / urine)	Yes	9	6	0.57[b]	1.41 (0.47- 4.21)
	No	93	44		
Sleeplessness	Yes	17	6	0.45[a]	0.68 (0.25- 1.85)
	No	85	44		
Depression	Yes	9	3	0.75[b]	0.66 (0.17 – 2.55)
	No	93	47		
Language comprehension deficits (aphasia, foreigner)	Yes	23	8	0.35[a]	0.65 (0.27- 1.59)
	No	79	42		
Memory impairment	Yes	6	1	0.43[b]	0.33 (0.04 – 2.79)
	No	96	49		
Dressings (sutures, decubitus wounds, etc.)	Yes	8	6	0.39[b]	1.60 (0.52 – 4.90)
	No	94	44		
Drainage	Yes	1	1	0.55[b]	2.06 (0.12- 33.65)
	No	101	49		
Urinary catheter	Yes	23	12	0.84[a]	1.08 (0.49- 2.41)
	No	79	38		
Therapy i.v. (peripheral or central venous access)	Yes	70	34	0.94[a]	0.97 (0.47- 2.01)
	No	32	16		
Therapy with sedatives	Yes	35	17	0.97[a]	0.99 (0.48 – 2.01)
	No	67	33		
Treatment with laxatives	Yes	11	8	0.36[a]	1.58 (0.59 – 4.20)
	No	91	42		
Diuretic therapy	Yes	30	13	0.63[a]	0.83 (0.39 – 1.78)
	No	71	37		
Antihypertensive therapy	Yes	60	32	0.58[a]	1.21 (0.60 – 2.45)
	No	41	18		
Substances Abuse (alcohol and drugs)	Yes	8	4	0.99[b]	1.02 (0.29- 3.57)
	No	94	46		
Patient in postoperative / anesthesia	Yes	2	1	0.99[b]	1.02(0.09 – 11.53)
	No	100	49		
Use of restraint means	Yes	6	12	**0.001[a]**	5.05 (1.77 – 14.43)
	No	96	38		

Bold: $p < 0.05$

BP: blood pressure

i.v therapy: intravenous therapy

(11, 22%), and the most part of controls showed 3 (28, 27.5%). In 4 cases (8%) and 5 controls (4.9%) were found 6 comorbidities in addition to principal diagnosis. The differences were not statistically significant (p= 0.334).

The two groups, cases and controls, were homogeneous for gender, age, citizenship, while, with respect to the parameters WHO, the adverse event *fall*, seemed to be associated with compiling Conley scale at the admission to care unit (OR = 4.92, 95%CI: 2.34 - 10.37) (Tab. II a, b).

Univariate analysis showed (Tab. IIa-IIb) that intrinsic factors that increase the risk of falling out are: presence of dizziness (OR = 3.22; 95%CI = 1.34 to 7.75), and psychomotor agitation (OR = 2.61; 95%CI = 1.06 to 6.43); among extrinsic risk factors the use of means of containment is associated with risk of adverse event (OR = 5.05 95%CI = 1.77 to 14.43).

Conditional logistic regression included '*risk of being a case*' as dependent variable, and the following covari-

Tab. III. Conditional logistic regression model of the association between event fall and covariates: psychomotor agitation, dizziness and means of restraint.

Covariates	HR	95%CI	
		Lower	Upper
Psychomotor agitation	1.085	0.282	4.169
Dizziness	3.966	**1.221**	**12.886**
Means of restraint	5.537	**1.288**	**23.798**
Model -2 Log likelihood: 79.33 Initial Log Likelihood function: -2 Log likelihood: 95.55 Likelihood ratio test: chi-square=16.220, df=3, **p=0.001**			

Bold: $p < 0.05$.

ates as independent: gender, age, dizziness, psychomotor agitation and use of means of restraint.
The fitted model (Tab. III) to analyze association between intrinsic and extrinsic factors and risk of falls, revealed a statistically significant association with the following outcome variables, in order: use of means of restraint (HR = 5.54, 95%CI = 1.29 to 23.80) and dizziness (HR = 3.97, 95%CI = 1.22 to 12.89). The variable psychomotor agitation is not significantly associated with the outcome fall (HR = 1.08, 95%CI = 0.28 to 4.17). The comparison of null model to the full model which includes the predictors shows a significant difference: the Likelihood Ratio Test is: $\chi^2 = 16.220$ with $p = 0.001$.

Descriptive analysis of falls dynamic

Fall events occur mostly during evening and night hours, tending to double in specific time range (2:00-2.30; 4:00-4:30; 6:00-7:00; 21:30-22:00) up to three times between 1:30-2:00 a.m. Other patients were present in 76% of cases (32/42), when event fall occurred, in 7.1% (3/42) of cases health personnel and in 2.3% (1/42) family members.
Modality of falling were, in order: from standing position in 42% of cases (18/43), from bed in 37% (16/43) of cases, by sitting in 14% (6/43) and from wheelchair in 2% (2/43).
With regard to reason of fall, 37% of falls (11/30) occurred as a result of loss of balance and sliding on dry pavement; while 13% (4/30) was due to loss of strength and 7% (2/30) was related to slipping on wet floor and stumbling.
Analysis of the fall place revealed that 82% (37/45) of falls occurred in the hospital room, 15% (7/45) in the bathroom and 2% (1/45) in the hallway.
The type of shoe worn at the moment of fall was, in 45% (16/36) of the cases 'an open type', in 14% (5/36) closed or socks, and in 28% (10/36) patient was barefoot.
Means of protection were not in use in 36% of cases (16/44), instead they were present in 64% of cases (28/44).
57% (24/42) of patients fallen had been allowed to get up out of bed, while 43% (18/42) had been forbidden.
Drugs that lower blood pressure, as antihypertensive (22/43, 51%) and diuretics (11/43, 25%), have been confirmed as the most frequently used among cases, followed by benzodiazepines or CNS sedative (10/43, 23%), laxatives (3/43, 7%) and other drugs (20/43, 46.6%).
Falls had serious damage as outcome in 80% of cases (35/43), while 20% (9/43) hesitated in minor damage. Considering the total cases, the most part of them did not require further investigation than the clinical visit, after the event, though in 22% of cases (11/50) and in 11% (9/50) more detailed radiological diagnostic exams, CT/MRI and RX respectively, have been necessary. Other tests have been conducted in 14% of cases (7/50).

Discussion

Falls are common among hospital inpatients. Rates from 2.9-13 falls per 1,000 bed days have been reported [5]. Up to 30% of reported falls [23] may result in injury, including fracture, head and soft tissue trauma, all of which may in turn lead to impaired rehabilitation and co-morbidity [15, 24].
In the present study, despite the heterogeneity of settings, populations and risk factors, a small number of items, provided by Conley Scale, emerged as significant in relation to risk falling: psychomotor agitation and dizziness, among intrinsic risk factors, already provided by Conley Scale, and use of restraints. The first two items increase the risk of falling , once and three times, respectively, the use of instruments of restraint, considered as extrinsic risk factor, increases the risk of falls over 4 times. The absence of filling in Conley Scale, at the entrance to care unit, predisposes to the event.
The intake of more than four drugs, the critical clinical conditions, the weakness in the hips and the unstable equilibrium, have been confirmed as characteristics that increase the risk of falling. On the other hand, the presence of co-morbidities in addition to the main diagnosis, does not appear to increase the risk significantly.
With regard to the category of drugs, antihypertensive and benzodiazepines are the most widely used among cases.
There is small but a consistent association between the use of most classes of psychotropic drugs and falls; specifically, odds ratio for sedative/hypnotic use was 1.54 (95%CI, 1.40-1.70) Further adjustment for confounders, dosage, or duration of therapy are necessary [25]. In addition, a metanalysis that studied the relationships of falls and medications which included studies that examined both multiple and single risk factors found a significantly increased risk from psychotropic medications (OR = 1.7), Class 1a antiarrhythmic medications (OR = 1.6), digoxin (OR = 1.2) and diuretics (OR = 1.1) [26]. As regards the description of the fall, most of them occurs during night hours, inside the inpatient room, and they are due to loss of balance or force. In the 80% of cases no outcomes of damage were reported, such as to require further diagnostic tests, such as x-rays or tc.
Interaction and probable synergism between multiple risk factors, is probably important such as identifying risk factors. Several studies have shown that the risk of falling increases dramatically as the number of risk factors increases. Tinetti et al. reported that percentages of persons falling increased from 27% for those with no or one risk factors, to 78% for those with four or more risk factors [27].

Newitt et al. reported that the percentage of community living persons with recurrent falls increased from 10% to 69% as the number of risk factors increased from one to four or more [28].
As emerged from other study, profile of patient at risk of falling, is a person with mental status and mobility impairments [29]. Intrinsic risk factors were identified by Rawsky's review [30]: cognitive impairment/psychological status, acute/chronic illness and mobility, sensory deficits, fall history, and elimination, recent syncope episode and cognitive status [31, 32]. Rubenstein et al. [7] analyzed 16 studies and reported the following risk factors, in order: muscle weakness, history of falls, gait deficits, balance deficits, use of assistive devices, visual deficits, arthritis, impaired activities of daily living, depression, cognitive impairments, and age 80 years. In general, factors related to cognitive impairment, functional decline, and chronic disorders result in a higher risk of falls [33].
Extrinsic factors (e.g., environmental hazards or hazardous activities) are described as primary causes for approximately half of all falls [34]. In a review of 20 articles, Connell [35] found that environmental hazards (e.g., walking on slippery/rough surfaces, obstacles, inadequate light, or loose carpets) create conditions for trips or slips in elderly people who may already have multiple intrinsic risk factors for falls. Additionally, the risk from hazardous activities can be increased by behaviors (e.g., faller was hurried or inattentive, difficulty or discomfort during a task, or moving beyond limits of stability). Within inpatient facilities, commonly reported extrinsic factors are related use of bedrails, height and stability of seating (low toilets, wheelchair braking problems, "gerichairs," or portable commodes), and obstacles created by mobility aids (e.g., wheelchairs and walkers;). Finally, common locations for inpatient falls are resident rooms or bathrooms, with the falls often involving problems with ambulation and transfers [35-37].
Falls occurred most often in the patient's room when they were alone and unassisted while trying to get to the bathroom [37].
An effective and efficient fall prevention program should require quick, reliable, and valid fall risk screens to identify high-risk patients. In general, recommend criteria for choosing the most appropriate assessment tool for a specific setting should be: high sensitivity, specificity, and reliability [36]. Despite this, the specific instrument chosen might vary, depending on the setting and professionals engaged for filling. Nursing assessment scales seem the most appropriate approach for Acute Care Settings and extended care setting, where the majority of patients may be at high risk. A substantial number of fall risk assessment tools are readily available, most with evidence supporting their reliability and validity [36].
Frequent monitoring of drugs effects on patients is crucial, according to the most part of guidelines. Drugs with an increased need for monitoring, due to their important impact on the risk of fall are: antihypertensive and diuretics as it can cause hypotension and incontinence of urgency; hypnotics; hypoglycemic; neuroleptics because they can induce extrapyramidal disorders. The infusion therapy would take a break during the night rest, when possible.
For prevention, containment and risk of falls multifactorial interventions are required in hospital , which ensure evaluative information essential for all types of patients, and educational interventions should be tailored to the risk emerged. A particular attention should be paid to periodic environmental assessment targeted on possible risk factors assessed.
Needs of mobilization and ambulation requires special attention by the medical staff, it's important to educate patient and caregivers about the correct methods for handling and having a safe ambulation (eg. How to perform postural changes or movements from bed to chair, pass from sitting to standing without loss of balance).
Patients and caregivers should be trained about aids and facilities, in order to avoid their misuse: encouraging the use of shoes with not slippery soles; instructing patients to get up slowly; assisting patients during high risk transfers (eg. from ambulatory to corridor once or vice versa).
There is no scientific evidence in literature, that use of physical or pharmacological restraint protect patients from falls. Means of mechanical restraint can result psychological and physical adverse effects, direct and indirect. Restraint should be applied only when strictly necessary, supported by prescription or documented evaluations by the nursing care staff, after understanding causes and after taking all possible alternative care strategies, including relational interventions.
Pharmacological restraints (sedation) is acceptable when it represents an integral intervention to the therapy. Alternatively to restraint, following initiatives can be undertaken: increasing surveillance, modifying treatments, preferring oral feeding to parenteral or nasogastric tube, removing catheters and drains.
Further environmental changes could be: to increase the light in the room; to place a disoriented patient near a gatehouse; do not place bed rails; to create a peaceful environment; to keep the alarm-bell close to patient and answer immediately to calls; to use reality-orienting therapy (ROT) or other psychosocial interventions for involving patient in the conversation; to provide reference points (calendar, television, radio, clock); to use listening activities; to promote cognitive recreational and physical activities.
Finally, as Tinetti et al. argued in a previous study [37], all prevention interventions are aimed at increasing nursing awareness of patient risk and involving patient and caregivers: teaching them; promoting patient independence and decreasing use of restraints; and, finally, paying attention to patients with impairments or altered elimination patterns (incontinence, frequency, nicturia).
Our results bear out the evidence of scientific literature about Conley employment in assessing *fall risk*. Lovallo et al. comparing Conley Scale and Hendrich Risk Model [37], stated that Conley Scale gave sensitivity and specificity values of 69-49% and 61% respectively. The Hendrich Model [37] gave a sensitivity value of 45-76% and a specificity value of 71%. Conley Scale is more indicated for use in medical and surgical sectors on the strength of its high sensitivity and specificity, since its

specificity is very low it is deemed useful to submit individual patients giving positive results to more in-depth clinical evaluation [22]. Conley Scale doesn't investigate some important clinical characteristics of patient that can represent a risk, like: visual impairment [14] and sensor-motor functions, incontinence, asthenia, cognitive impairments or sedation [14, 27], and depression; likewise it is important to assess environmental conditions and the use of assistive devices, as well [22].

Conclusions

It has been shown that in acute care settings, falls during hospitalization are more common in confused patients and those with greater comorbidity [24]; but the present study doesn't support this evidence, maybe comorbidities should be stratified for disease severity.
Risk factors should be stratified for specific unit care departments, this population was heterogeneous for diagnosis and sample size was small. The most part of cases have been recorded in Neurology Department, and it is already acknowledged that several diseases have been shown to increase the risk of fall such as Alzheimer's disease [39], Parkinson's disease [28], and stroke [40].
According to the literature further studies need to be carried out in order to:

- test Conley Scale on a larger sample and stratify risk for unit department or comorbidities [41, 42];
- empower the role of nurses in fall prevention: encouraging the account of risk factors not provided by Conley, intrinsic and extrinsic; carrying out a comprehensive patient evaluation of motor cognitive functionality and psychological status [43];
- involve and inform patient and caregivers about fall risk.

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Appendix 1

DATA COLLECTION FORM HOSPITAL ACCIDENTAL FALLS

N…….

0.MEDICAL RECORD CODE …….

1. Is this patient fallen? ☐ yes (CASE) ☐ no (CONTROL)

2. Patient data:
2.a Birth date (dd/mm/yyyy)…… (age…… with respect to year of hospitalization)
2.b Gender: M ☐ F ☐
2.c City of residence……2.d County/district…….
2.e Nationality: ☐ Italian ☐ foreigner

3. Hospitalization data
3.a Date of admission: dd/mm/ yyyy:……../……./……..….
3.b Date of discharge: dd/mm/ yyyy:…..…../…..…./…….
3.c Department of Hospitalization:……..……..(see DB)
3.d Inpatient: < > ordinary < > day hospital
3.e Inpatient type: < > urgent < > planned admission (RRR into TRIAGE form)
< > transferred (see Fax from other hospital)
3.f Primary diagnosis code (ICD9-CM) : ……
3.g Secondary diagnosis codes : I …….II……..…III……..…IV……V……..……

4. Patient at admission
4.a Unstable/critical patient: yes ☐ no ☐
If not, answer to following 4 questions (**OMS factors**):
4.b .> = 65 years yes ☐ no ☐
4.c More than four drugs in assumption:yes ☐ no ☐ ß (see ED form or anamnesis)
4.d Hips weakness: yes ☐ no ☐
4.e. Unstable balance: yes ☐ no ☐
4.f Conley filled in at admission: yes ☐ no ☐
4.g. If CASE: Conley Scale filled in available before fal: yes ☐ no ☐
4.h Data. Date of the last available CONLEY Scale (FOR CASES: Conley <u>before the fall</u>):
……/……./…….(dd/mm/aaaa)

Insert Data of CONLEY mentioned above:

C0. Questions to :	Patient ☐	Caregiver ☐	Familiy ☐
Previous Falls			
	yes	no	missing
C1. Have you fallen in the last three months?			
C2. Have you never had vertigo and dizziness? (In the last 3 months)			
C3. Have you ever lose urine or feces while going to the bathroom? (In the last 3 months)			
Cognitive impairment (Nurses' Observation)			
C4. Impaired walking, creeping step, broad base of support, unstable march			
C5. Agitated (definition: excessive motor activity, usually no purposeful and associated with internal tension. Ex. Inability to sit still, moving restlessly, pulling clothes, etc.)			
C6. Impairment of judgment / lack of a sense of safety awareness			

CONLEY INDEX ≥ 2 Nursing diagnosis: patient at risk of falling

5. Factors present in the day of case fall:
Fill in with 'x' for cases and for controls, referring to the day of fall event:
YES, if the features are explicitly mentioned in the medical record.
NO, if the features are explicitly mentioned in the medical record.
NOT PRESENT, if they are not reported in the medical record.

Body Functions	yes	no	NOT PRESENT
F1) Hypotension: PA systolic <= 90			
F2) Hypertension: PA systolic> = 180			
F3) Fever >=38° (vedi diario)			
F4) unhealthy state of consciousness			

Fill in for cases and controls referring to the day of the fall of the case.
Mark with a "x" in case of presence inside medical record or in case of element otherwise deductible for clinical condition.

INTRINSIC FACTORS	✓
FI1) Disoriented	
FI2) Presence of Dizziness	
FI3) (alias C4) Impaired walking, creeping step, broad base of support, unstable march	
FI4) (alias C5) Agitated (definition: excessive motor activity, usually not finalized and associated with inner turmoil. Ex. Inability to sit still, moving restlessly, pulling clothes, etc.)	
FI5) (alias C6) Impairment of judgment / lack of a sense of danger	
FI6) Reduced muscle strength	
FI7) Patient with impaired vision	
FI8) Incontinence (fecal / urine)	
FI9) Insomnia (see Drugs Benzodiazepines / Diary)	
FI10) DEPRESSION	
FI11) DEFICIT UNDERSTANDING LINGUSITCA only if explicitly stated (aphasia, stranger)	
FI12) Memory impairment only if explicitly stated	
Extrinsic factors	
FA1) Dressings (sutures, decubitus wounds, etc.) (see Diary / Register operative)	
FA2) Drainage (see Diary and kind intervention)	
FA3) Catetere urinario (vedi Diario)	
FA4) Therapy i.v. (peripheral or central venous access) (see chart therapy)	
FA5) Therapy with sedatives (see data falls for cases)	
FA6) Laxative therapy (see data falls for cases)	
FA7) Diuretic therapy (see data falls for cases)	
FA8) Antihypertensive therapy (see data falls for cases)	
FA9) Substance Abuse (alcohol and drugs) (see Diary)	
FA10) Postoperative patient / anesthesia	
FA11) Use of instruments of restraint	

Fill in Only for Cases Module: Fall Form

6. Index of risk fall at admission (Conley: last value calculated before the fall)…….

7. DAI…….8.UO(Operative Unit)……

Nursing Section

9. Date of the event ……... 10. **Time**……(hh:mm)

11. WHO WAS PRESENT:
☐ alone ☐ other patients ☐ family ☐ health staff ☐ else……

12. MODE OF FALL:
☐ from a standing position ☐ by sitting ☐ from bed ☐ from wheelchair
☐ during transfer ☐ else ☐……..

13. REASON:
☐ loss of strength ☐ loss of balance ☐ loss of consciousness ☐ stumbled ☐ slipped, dry floor ☐ slipped, wet floor ☐ unknown ☐ else
……..…..

14. PLACE:
☐ room ☐ aisle ☐ bathroom ☐ stairs ☐ surgery ☐ outside ☐ else …….

15. WHAT WAS THE PATIENT DOING WHEN FALLEN? .....

16. TYPE OF SHOE: ☐ open ☐ closed ☐ barefoot ☐ socks

17. PROTECTIVE EQUIPMENT IN USE: no ☐ yes ☐ **Specify**…….

Medical Section

18. DIAGNOSIS AT THE ENTRANCE …….

19. PATIENT ALLOWED TO GET UP: ☐ No ☐ Yes

20. FALL Results:
☐ absence of apparent damage ☐ minor injury: bruising or abrasion
☐ moderate damage ☐ major damage ☐ death

21. P. A. in supine…….. **and orthostatic**……**(if possible)**

22. DIAGNOSTIC TESTS REQUIRED:
☐ none ☐ TC/RMN…… ☐ RX…… ☐ else……..

23. THERAPY IN ACT: ☐ CNS sedative ☐ laxatives ☐ diuretics ☐ antihypertensive ☐ else……

24. Prognosis: ☐ none ☐ sligh ☐ moderate ☐ severe ☐ serious ☐ death

ORIGINAL ARTICLE

Geocoding health data with Geographic Information Systems: a pilot study in northeast Italy for developing a standardized data-acquiring format

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Key words

Environment and Public Health • Geographic Information Systems • Data matching

Summary

Introduction. *Geographic Information Systems (GIS) have become an innovative and somewhat crucial tool for analyzing relationships between public health data and environment. This study, though focusing on a Local Health Unit of northeastern Italy, could be taken as a benchmark for developing a standardized national data-acquiring format, providing a step-by-step instructions on the manipulation of address elements specific for Italian language and traditions.*

Methods. *Geocoding analysis was carried out on a health database comprising 268,517 records of the Local Health Unit of Rovigo in the Veneto region, covering a period of 10 years, starting from 2001 up to 2010. The Map Service provided by the Environmental Research System Institute (ESRI, Redlands, CA), and ArcMap 10.0 by ESRI® were, respectively, the reference data and the GIS software, employed in the geocoding process.*

Results. *The first attempt of geocoding produced a poor quality result, having about 40% of the addresses matched. A procedure of manual standardization was performed in order to enhance the quality of the results, consequently a set of guiding principle were expounded which should be pursued for geocoding health data. High-level geocoding detail will provide a more precise geographic representation of health related events.*

Conclusions. *The main achievement of this study was to outline some of the difficulties encountered during the geocoding of health data and to put forward a set of guidelines, which could be useful to facilitate the process and enhance the quality of the results. Public health informatics represents an emerging specialty that highlights on the application of information science and technology to public health practice and research. Therefore, this study could draw the attention of the National Health Service to the underestimated problem of geocoding accuracy in health related data for environmental risk assessment.*

Introduction

In the past decade, Geographic Information Systems (GIS) have become an innovative and somewhat crucial tool for analysing relationships between public health data and environment. According to Longley et al. (2005), a GIS is an application-led technology, which can be used, in this instance, for monitoring and understanding observed spatial distribution of attributes such as the geography of environmental health [1]. Thus, it is required to transpose data stored in a health care related database to a spatial related database, assigning to each record a univocal spatial location (X, Y Coordinates). This procedure is known as geocoding. Its role continues to grow and evolve as new forms of geocoding emerge and as geocoded data are applied to an ever-diverse set of spatially based investigations [2]. Geocoding technology has been applied in many fields: social, political, and economic and more recently in public health research and practice. In general, these applications are related to interpolation rather than matching. This is because interpolation requires a lower level of accuracy in data manipulation. Clustering, aggregation, spatial smoothing are typical applications in epidemiology. The literature provides many examples focused on the surveillance of infectious and chronic diseases [3-5], environmental exposures [6, 7], drinking water epidemiology [8, 9] and pharmacoepidemiology [10].

In general, the limitation is the spatial accuracy of the geographic location computed for any particular subject. Accuracy represents an important issue particularly in Italy due to the complexity of the address name and street morphology. Address complexity and street morphology depend on historical heritage of Italy, so georeferencing requires additional manipulation of place names data. In this scenario, the improvement of geocoding accuracy plays a key role in developing a reliable tool for public health research in Italy. The main aim of this paper is to describe the procedures involved in the conversion from database collected data into geocoded dots representing a health event, in order to display the spatial distribution of five major chronic-degenerative diseases within the Rovigo Local Health Unit (ULSS N.18) in the Rovigo

Province (Veneto Region - North Italy). The highly accurate geographic localization of patients, served by the Local Health Unit, will widen the range of opportunities for further spatial analysis and modelling, such as environmental related hazard or monitoring the prevalence of certain diseases through time, gender or age. This paper therefore provides a step-by-step instructions on the standardization of address elements specific for Italian language and traditions.
A description of the methodology involved in the geocoding process will be as crucial as outlining some guidelines for a standardized method, strongly required for the data collection component, involving local addresses. This study, though focusing on the Local Health Unit of Rovigo, could be taken as a benchmark for developing a standardized national data-acquiring format.

Methods

The Study Population

The Local Health Unit of Rovigo (ULSS N.18) has collected data on their catchment area, made up of 41 municipalities, over a period of 10 years, starting from 2001 up to 2010, and stored it in Microsoft *Access*™ database format, for 268,517 records (Fig. 1).
It is crucial to understand that 268,517 is the total amount of records collected in the above-mentioned period, while the population census, provided by the National Institute of Statistics (ISTAT) at 1st of January 2011 was 175.816 persons residing in those 41 Municipalities.
For this study's purpose, the year 2010 has been chosen as sample group, hence the provided data had to be checked and sorted carefully. By means of SQL query language, a sequence of selection criteria has been applied on the original database and the amount of valid records significantly shrank, from 268,517 to 178,183. This decrement is ascribed to the number of subjects whose status was 'deceased' or 'transferred' to a different ULSS up to the 31st of December 2009, as well as to those subjects who have their domicile outside the ULSS of Rovigo. To obtain a consistent sample, it has been decided to remove those subjects having a residence address within the ULSS of Rovigo, yet having their permanent address outside of it. Forthcoming analysis will use this data for mapping and clustering health events associated to environmental hazards, therefore it is assumed that domicile related data has a greater deal of truthfulness compared to residence data [11]. The difference of 2,367 subjects between the census data and the collected data lies in the amount of persons residing in a different municipality, though attending to the Local Health Unit of Rovigo.
For personal data protection policy, a progressive sequential unique identifier, linking to a different database, has replaced all information regarded as sensible data. Further information stored were gender, date of birth and mostly important, an alphanumerical code (Tax Code) for personal and unambiguous identification issued by the National Health Service (NHS). Moreover, a numeric code is included, which identifies the current status of patients within the Local Health Unit (LHU), for instance, if the patient is active, transferred to a different ULSS or dead.

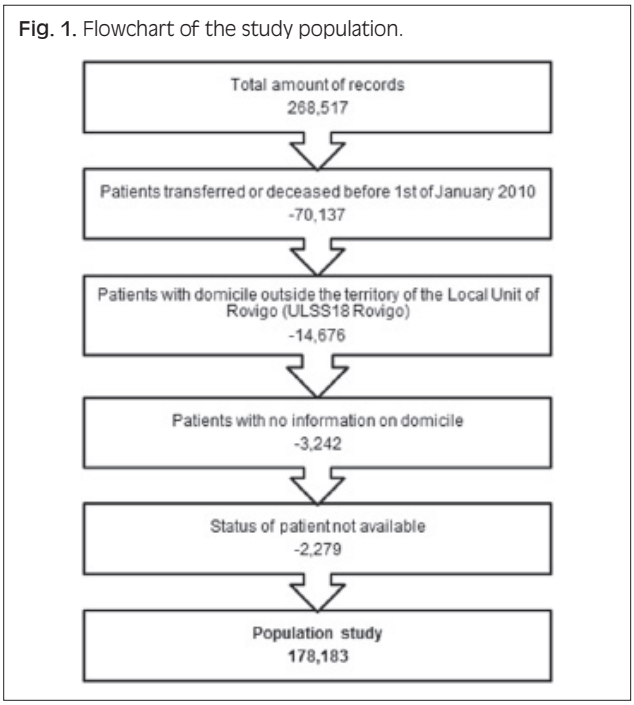


Fig. 1. Flowchart of the study population.

The Geocoding Process

The geocoding process involves converting a string information, such as a street names, town or place name, into geographic features located on the earth's surface, which can be spatially displayed within a GIS. Finding good quality up-to-date reference data becomes a crucial point, hence different commercially available street network databases have been weighed. The Map Service, named World Street Map 2010, provided by the Environmental Research System Institute (ESRI, Redlands, CA), and ArcMap 10.0 by ESRI® were, respectively, the reference data and the GIS software, employed in the geocoding process.
Within the tools available for geocoding, the Geocode Address Tool was implemented as it allows for the geocoding of a table of addresses. However, in order to match the addresses in the input table, this tool needs to link to the reference data using a service provided by the ESRI Address Locator. It was opted for the TA_Address_EU.GeocodeServer locator, specific for the European Zone, where the domicile address is parsed into 4 syntactic components. The fields required by the operation are:

1. Address: Street name with suffix type (e.g. road, avenue, boulevard) and house number.
2. City: the name of the municipality.
3. Postcode: a 5 number code (related to one or more Municipalities).
4. Country: the code of the country of domicile, in this instance ITA.

The result of a geocoding process is an output table returning the addresses with a score of the probability of

Tab. I. Tools employed for manual localization of addresses.

Web Map Service (WMS)	Google Maps Google Street View	http://maps.google.it/ http://maps.google.it/	GoogleMaps - ©2012 Google GoogleMaps - ©2012 Google
	VirgilioMappe	http://mappe.virgilio.it/	Matrix® S.p.A.
	Tuttocittà	http://www.tuttocitta.it/	Navteq© Ximage®
Online Telephone Directories	White Pages	http://www.paginebianche.it/	Seat Pagine Gialle© S.p.A.
	Yellow Pages	http://www.paginegialle.it/	Seat Pagine Gialle© S.p.A
	Pronto Comune	http://www.prontocomune.it/	Società Editrice Europea® Srl
Online City Maps	Geoplan	http://www.geoplan.it/	Geoplan© S.r.l.

having matched the correct location. In fact, geocoding is a probabilistic system, where each field participating in the linkage comparison is subject to error and is measured by the probability that the field agrees versus the probability of chance agreement of its values [11]. Consequently, two fields are generated in the output table showing the match type and the score: the former indicates whether there was a match (M), an unmatched result (U), or tied results (T), which requires to be manually checked by the operator. On the other hand, the score is expressed as the percentage of having identified the best possible candidate of the address within the reference data.
For the manual localization of those addresses not matched automatically, a wide range of open source resources have been employed (Tab. I).

Results

The first attempt of geocoding produced a poor quality result having about 60% of the addresses tied while only 40% matched. After having manually verified those records it was noticed that although the street name was present in both, the reference data and the input table, it was spelled the other way round. A procedure of manual standardization was performed in the input table, so that all the streets name were spelled correspondingly to those in the reference data.
After carrying out the previously mentioned adaptations, the result of the geocoding had significantly improved, reaching almost 98% of matched (M) records and 2% of tied (T) records, yet this outcome does not reflect the real precision of the result. In fact, as previously explained, the address is parsed into 4 components, and a match (M) result is achieved every time just 2 of these are met by the query. As a result, 3 different levels of matching precision have been identified, depending on the number of the address components available during the geocoding process. Therefore, when merely the City and Country fields are matched the M type is specified as EU_City.ITA; likewise EU_PostCity.ITA identifies those records where only the Postcode and Country proved to correspond, whilst EU_StreetName.ITA refers to those record matched at a street name level.
As shown in Table II, the percentage of EU_StreetName.ITA addresses matched is 90.9% while the addresses geocoded at a city and postcode level are respectively 0.18% and 6.8%.

Tab. II. The percentage of addresses geocoded according to match type.

Match type	Match level	Match score (%)	No. of addresses	% of addresses
U - (Unmatched)	none	none	none	
T - (Tied)	EU_City.ITA	100	24	0.01%
T - (Tied)	EU_Street_Name.ITA	≤ 69 70-99 100	423 45 3,137 **3,605**	2.02%
M - (Matched)	EU_City.ITA	≥ 90	333	0.18%
M - (Matched)	EU_PostCity.ITA	≤ 99 100	137 11,981 **12,118**	6.8%
M - (Matched)	EU_Street_Name.ITA	≤ 69 70-79 80-89 90-99 100	3,859 2,885 3,038 1,100 151,221 **162,103**	90.98%
			Total 178,183	100%

Tab. III. Parcelling of the addresses for geocoding.

Attribute Name	Street Prefix	Street Name	House Number	Unit Number	Postcode	Municipality Code	Municipality Name	Country Code
Example	Borgo Contrada Corso Galleria Largo Località Piazza/Piazzale Via/Viale Vicolo Villaggio Strada Zona Etc..	Title (if available: grade or clerical rank with no abbreviation) + space key + Name (in extenso) + space key + Surname No article No preposition No apostrophe	House number of the Building	Apartment or sub-unit number in Arabic Numeral or Roman Numeral or Letters of the Alphabet	Postal Code (ambiguous) Five numbered code	Provided by the National Institute for Statistics (Istat) (unambiguous) Five numbered code	Name of the Municipality (in extenso)	ITA
Data Type	String	String	Short Integer	String	Short Integer	Short Integer	String	String
Geocoding elements	✓ (as a single string)		✓	×	✓	×	✓	✓

Successively, addresses matched at a street name level, were weighed against the score achieved during geocoding. Locations that yield a score of ≥ 90 were considered a good match whilst those with score ≤ 89, approximately 9,700 records, required be checking individually and adjusting by hand. Though assuming the correctness of those 151,221 results having a match at a street name level and a score ≥ 90, it was opted to verify if the addresses did actually coincide with the true location on the map. For this purpose, the Municipality of Rovigo was chosen as the sample unit, since it is the largest municipality with the highest number of people attending to the LHU. The 32% of the above mentioned addresses, that is roughly 48,000 patients, fall within the administrative boundaries of Rovigo and their geometry has been checked using the Intersect tool of ArcInfo®.

The point feature class, representing the patients' domicile, was intersected with the line feature class of all the road segment attributes, and a new point feature dataset was generated which includes, for each address, the name of the street segment that was overlapped. After carrying out a SQL query on 48,615 records, as many as 9,165 patients appeared to have the domicile address matched to the wrong street segment. However, after a double-check it was realized that differences were caused mainly by the presence/absence of the apostrophe, article or capital letters in the street's name, yet the correctness of the match was not compromised. Only 324 records, equal to the 0.7%, were wrongly matched since the error was caused by the street names, in the line feature class, being more up-to-date than the address associated to the patient's domicile stored in the LHU database.

On the other hand, a procedure of manual geocoding had to be carried out for those addresses with a city or postcode level match (M), for a total amount of 12,451 patients. That is to say, the geocoding process was not able to assign a street segment to the address, therefore positioned the patient's location at the geometric center of the Municipality. This entailed to seek for the correct coordinates by means of several web mapping service applications, online telephone directories and, in the most difficult cases, even by contacting the Municipality office.

Furthermore, all results with a tied (T) match type were assessed individually to remove any uncertainty; for this, only 447 proved wrongly geocoded and the right coordinates have been assigned manually.

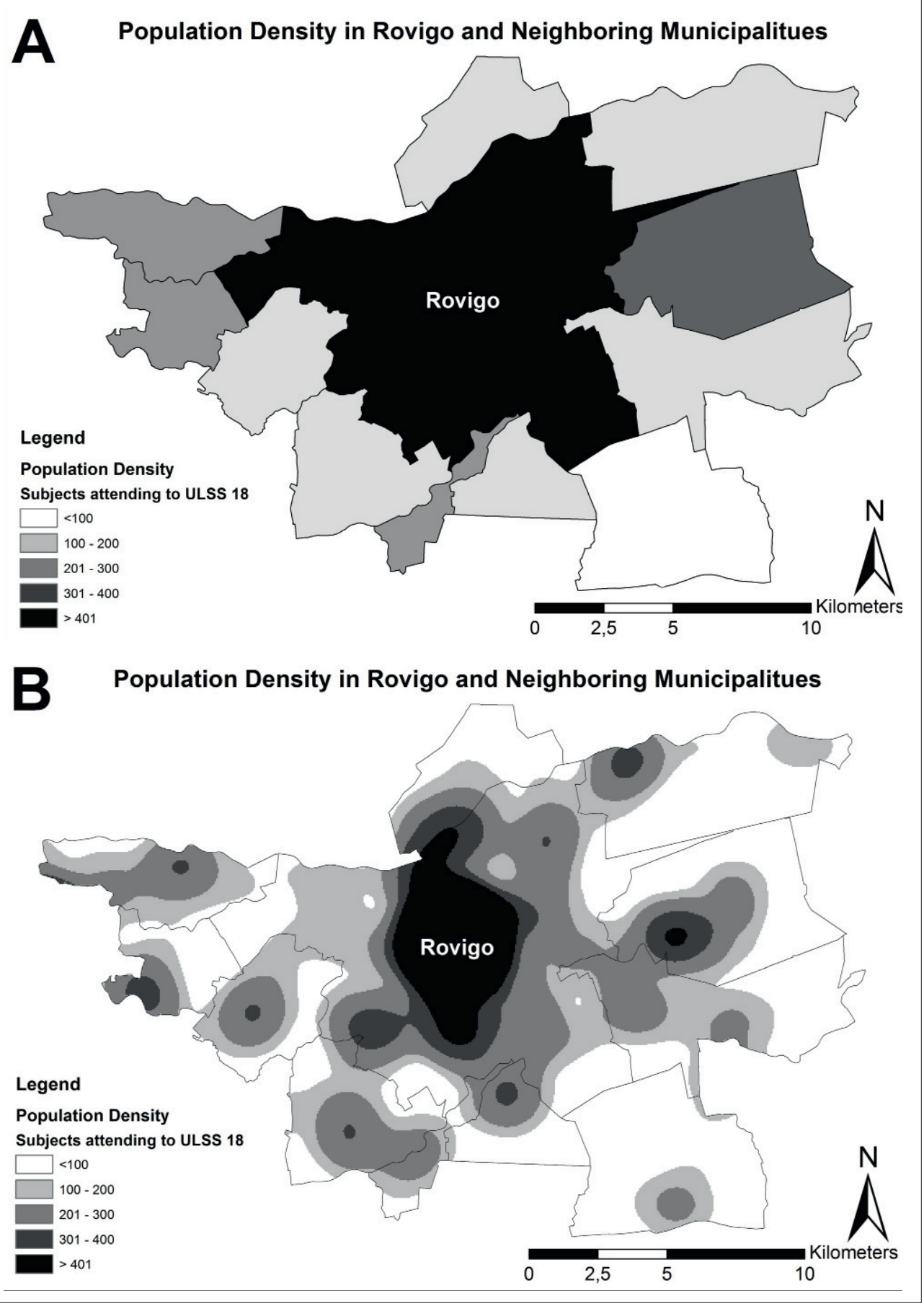
The guiding principles, which should be pursued for preparing data for geocoding, are summarized and exposed in Table III. These, however, do referred strictly to the ESRI World Street Map web service, used as the main reference data, to which addresses should conform.

In a health related prospect, the result of having a high detail geocoded population could increase the perception and comprehension of the distribution of any given health event, thus allowing for an administrative boundless view. As showed in Figure 2, the population density can be represented with neat lines, according to the Municipality geographical layout (Fig. 2 part A), or boundless, that is to say representing any event, in this case the permanent address of the case study subjects, with no constrains associated to human made frontiers (Fig. 2 part B). This instance could be applied when mapping the spatial distribution of some chronic-degenerative diseases, such as asthma, and analyzing if a given pattern could be linked to an environmental hazard, such as air pollution or the proximity to dumps, industries, incinerator, etc.

Discussion

Ecological studies are not based on individual but on aggregated disease and exposition data [12]. The prospect of a wide range of spatially related Health analysis, such as disease clustering and risk exposure to environmental hazards, on a large number of patients, was the leading endeavor of this project. Out of the 286,517 records pro-

Fig. 2. Difference between geocoding founded on a municipality boundary basis and a street level basis.



vided by the Local Health Unit of Rovigo, 178,183 had the prerequisites for being assessed in this study, having lost 2% due to void entries in the database and 31.6% of the data being irrelevant for the study area.
The standardization procedures of the address database, produced a result of 162,103 matched and 3,605 tied addresses, both at a street name level, equal to the 93% of all records. For the remaining 7%, that is 12,475 addresses, matched or tied at a city or postcode level, the geocoding had to be performed manually by the operator, using a wide range of open source data and, if necessary, with the aid of the Municipalities involved.
The geocoding process revealed a dearth of homogeny between the address labeling used by the Local Health Unit, during the registration procedures of patients, and the label attributes of the street segments in the reference data. In particular, there was a conflicting approach in using abbreviations for streets named after Saints, clergy characters and military ranks, as well as the reverse writing of historical figures names, for instance, surname-name order in the input data and name-surname order in the reference data. Furthermore, streets named in memory of historical dates were written in Arabic numeral and in Roman numeral, respectively.
Overall, a lack of consistency in the approach of storing personal data has emerged. In particular, the street prefixes were stored with a variety of abbreviations leading to ambiguity.
As far as the street name is concerned, the name and the title, when included, should not be shortened, as it will result in misspelling errors or in homonymy. Furthermore, neither article, nor preposition, nor apostrophe should be included.
With respect to the municipality details, a few points should be considered: firstly, the name should be written in full length to avoid false mismatch and, secondly, it should always be coupled to the postcode. Unlike in the United States, where the U. S. Postal Service (USPS) uses a zoning improvement plan (ZIP) code as a postal addressing standard [13], in Italy the post code does not serve as an unambiguous identifier, hence more than one municipality can have the same post code.
Health data should be collected originally with compliance to a set of well-defined parameters, if possible using a menu-driven interface with drop-down lists to facilitate users by selecting among a list of pre-compiled values. Misspelling errors of streets, for instance, could be reduced considerably, as well as gross inconsistencies between the municipalities' name and postcodes. The National Health Service (SSN) should consider acquiring a common program and standardize parameters to collect health data.
The main achievement was to outline some of the difficulties encountered during the geocoding of Health data and to put forward a set of guidelines that could be useful to facilitate the process and enhance the quality of the results.
On the other hand, some limitations of this study should be considered. First, given the massive amount of records that had to be geocoded, it was opted to ignore the house number, as it would introduce an additional time consuming and labor-intensive effort to locate manually the wrongly matched addresses. Georeferencing with street centerline data can affect location accuracy, since it introduces many assumptions including the equal parsing of addresses along a road network and the uniform distancing of houses from the road network [14].
Second, there was no possibility to account for the positional accuracy of the results obtained by the use of the ESRI StreetMap as reference data. Accordingly to Zhan et al. (2006), the validity of epidemiologic research depends on the match rate of geocoding (the percentage of addresses geocoded), as well as the positional accuracy of locations of geocoded addresses. Thus, in this study it was not possible to calculate the positional accuracy, defined as the difference between the geographic location of a geocoded address and the ''true'' ground location of that address determined by using a field survey method, i.e., surveying using a global positioning system (GPS) device [15].
According to a recent study, the current state-of-practice lacks of standard resources for geocoding, geocoding accuracy assessment, and for evaluating the impacts of geocoding error on public health decisions [16]. Even though in the last decade several studies have been carried out on the accuracy of geocoded data [14, 17, 18]. No studies have addressed the completeness and accuracy of the reference street network database in Italy.
As a matter of fact, no research has been found in literature which evaluates the topic of geocoding methods in this country, although implementing address coded data in epidemiology research is becoming rather frequent [19, 20].
The research project will now proceed by evaluating risk exposure to environmental hazards, for instance air pollution, and the spatial distribution of some chronic-degenerative diseases, such as asthma, linking health data to the georeferenced patients in the Local Health Unit of Rovigo. Forthcoming results will be soon expounded.

Conclusions

The main achievement of this study was to outline some of the difficulties encountered during the geocoding of Health data and to put forward a set of guidelines that could be useful to facilitate the process and enhance the quality of the results. Health data should be collected originally with compliance to a set of well-defined parameters, if possible using a menu-driven interface with drop-down lists to facilitate users by selecting among a list of pre-compiled values and avoid misspelling bias. Public health informatics represents an emerging specialty that highlights on the application of information science and technology to public health practice and research. Therefore, this study could draw the attention of the National Health Service of Italy to the underestimated problem of geocoding accuracy in health related data for environmental risk assessment.

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ORIGINAL ARTICLE

Is individual social deprivation associated with adverse perinatal outcomes? Results of a French multicentre cross-sectional survey

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Key words

Adverse perinatal outcomes • Preterm birth • Individual Social deprivation • EPICES score

Summary

Introduction. *French national health programmes take into account social deprivation in their implementation, those targeting perinatal outcomes, especially. The main aim of the present work was to assess the association between individual social deprivation and adverse perinatal outcomes.*

Methods. *A multicentre cross-sectional population-based survey was performed between October and December 2007. Eligible women delivered a baby in one of the three maternity hospitals of Clermont-Ferrand area, and read and spoke French fluently. Women who had undergone voluntary termination of pregnancy were excluded. Individual social deprivation was measured by the EPICES score. Standard prenatal follow-up defined by having less than 7 consultations and quality of prenatal care defined by having at least four consultations were measured. Adverse perinatal outcomes were measured by a composite criterion defined by women who had the occurrence of the three main causes of pregnancy-related disorders: preterm delivery, and/or diabetes, and/or obstetrical hypertension.*

Results. *Of the 471 eligible women, 464 were finally included. One hundred and fifteen (24.78%) women were socially deprived. The most deprived women had poor standard prenatal follow-up ($p = 0.003$) and poor quality of prenatal care (0.03). Nationality was the sole confounding factor identified. Deprived women had a two-fold greater risk of adverse perinatal outcomes, adjusted odds ratio 1.95 [1.15; 3.29].*

Discussion. *Social deprivation was associated with adverse perinatal outcomes. Social deprivation should be systematically screened in pregnant women standard follow-up, among migrant women, especially.*

Introduction

The process of deprivation was defined first by J. Wrezinski and P. Townsend who also reported that deprivation is the main cause of inequalities in health [1-4]. The French High Committee on Public Health reports of 1998 revealed inequalities in health among the most deprived and pregnant women especially, with lower follow up and higher adverse perinatal outcomes as preterm delivery [5, 6]. Several studies have already shown an association between socioeconomic deprivation and adverse birth outcomes [7-16]. Whole of those studies measured deprivation by using geographical indices limited by "the ecological bias" [17, 18]. Actually, four French surveys dealt with individual social deprivation and adverse perinatal outcomes [19-22].

The Europeristat report on perinatal health indicators published in 2010 revealed that the French early and late neonatal mortality rate per 1,000 live birth was 2.4 ranking France at seventeenth. It also showed that maternal mortality rate in 2006-2010 was 8.4 per 100,000 live birth far from the Sweden rate at 3.1. Considering those results, a French national perinatal program was developed between 2005 and 2007 aiming to improve access to perinatal health care service for the most deprived pregnant women and to reduce maternal and perinatal morbidity and mortality by 40% and 15%, respectively. National guidelines concerning prenatal care of pregnant women and the importance of identifying those in a vulnerable situation have been developed by the French National Authority for Health in 2007 (http://www.has-sante.fr).

The main aim of the present work was to assess the association between individual social deprivation and adverse perinatal outcomes.

Methods

Background

In 2009, the urban area of Clermont-Ferrand had 259,702 inhabitants and three maternity hospitals, two public of level III with obstetric, neonatology and neonate recovery units and one private of level II with obstetric and neonatology units. These maternity units were coordi-

nated by the perinatal network of Auvergne (Réseau de Santé Périnatale Auvergne-RSPA). In 2011, 2,857 births were registered.
French pregnant women follow-up has been established by the decree number 92-143 of February the 14th, 1992. Women with low-risk pregnancies undergo a standard prenatal follow-up of 7 consultations (one before the end of the third month and one visit a month), and three ultrasound examinations (one between 11 and 13 weeks, one between 20 and 24 weeks, and one between 30 and 35 weeks). The French national health insurance fund reimburses this follow-up.
In France, health examination centres, providing free medical consultations to recipients of the national health insurance for salaried workers, developed in 2002 a reliable individual score of deprivation, called the EPICES (Evaluation de la Précarité et des Inégalités de santé dans les Centres d'Examens de Santé - Evaluation of Deprivation and Inequalities in Health Examination Centres) [23-26]. This score is composed by 11 items on marital status, health insurance status, economic status, family support and leisure activity during the last 12 months (Appendix 1). It has been validated on a cohort of 197,389 persons [19, 27, 28]. The EPICES score is computed by adding each question coefficient to intercept whenever the answer is "yes". The higher the score, the more deprived the women is. A reliable threshold of 30.17 was previously identified with deprived persons having a score equal to or above 30.17 [19].

Patients

Eligible women delivered a baby in one of the three maternity hospitals of Clermont-Ferrand area and had a fluent command of spoken and written French. Women who delivered a foetus by medical abortion were not included in the study.

Methods

A multicentre cross-sectional population-based survey was carried out between October, the 25th, and December the 27th, 2007.
Sociodemographic and medical data was collected in women medical record by healthcare professionals or during the interview performed by the research team. The interview was performed during the hospital stay, at the postpartum period, by a member of the research team.
Data was gathered about socio-demographic status, family status; couple vs. single (women not married or not living with a partner) and professional status; employed, unemployed, parental leave and other (pupil, student, and trainee). Medical and obstetrical data was collected, including parity (number of children the women had, excluded the current delivery), obstetrical history of induced abortion (voluntary termination of pregnancy before 14 weeks), miscarriage (spontaneous abortion < 22 weeks), and caesarean section. Then, labour and delivery characteristics, post-partum haemorrhage (> 1 litre), birth weight and percentile, calculated from gestational age and sex according to the AUDIPOG curves (http://www.audipog.net/) and five-minutes Apgar score were gathered.
Standard prenatal follow-up defined by 7 consultations and quality of prenatal care defined by at least four visits during pregnancy were measured.
The EPICES score was calculated for each women included in the survey and ranked in the deprived category when it was equal to or above 30.17.
Adverse perinatal outcomes were measured by a composite criterion. This criterion included the three main causes of pregnancy-related disorders: preterm delivery (< 37 weeks of gestation), gestational diabetes and high blood pressure during pregnancy (gestational hypertension, pre-eclampsia, and eclampsia) (www.cngof.asso.fr). The criterion was quoted 1 if women had preterm delivery, and/or gestational diabetes, and/or high blood pressure during pregnancy.
All participants gave their informed consent to be enrolled. Data coming from computerized medical records were reported to the French National Commission for Data Protection (CNIL- Commission Nationale de l'Informatique et des Libertés) (N° 1268114). Under French law, this study was exempt from approval by an ethics committee.

Statistical analyses

Descriptive analysis assessed women's characteristics and prevalence of social deprivation. Bivariate analysis was performed by using Chi-square test or Fisher's exact test for qualitative variables and with Student t-test or Wilcoxon test for quantitative variables. First, association between EPICES score and the composite criterion was assessed. Second, relationship between EPICES score and explanatory variables associated with adverse perinatal outcomes in the literature; age, nationality, employment status, tobacco smoke during pregnancy and having medical history of induced abortion, was performed to identify variables that can be confounders. Third, the Mantel-Haenszel method was performed to identify and to consider variables being real confounding factors. A threshold of 10% was taken for the Mantel-Haenszel method to not neglect real confounding factors [29]. Then, a multivariate analysis was performed by using logistic regression that included EPICES score, real confounding factors identified and interactions between EPICES and confounding factors. Results of the logistic regression were presented trough the adjusted Odds Ratio (aOR) with their 95% confident interval (95%CI). All the other statistical analyses were performed with a meaningful threshold of 5%. Statistical analysis was performed on SAS software (V9.3. SAS Institute Inc., Carry, NC, 2002-2003).

Results

Among the 477 women who delivered a baby, 471 women were eligible and 464 (98%) were finally included; seven women did not gave their informant consent to be enrolled. Of the women included, (92%) were French, aged 29 years old (standard deviation 5.05) and 46% lived single. Deprived women were younger, migrants,

Tab. I. Descriptive and bivariate analysis of women's sociodemographic characteristics in the overall sample and according to the social deprivation status.

	Overall sample N = 464 %	Non-deprived N = 349 %	Deprived N = 115 %	p
Age (years)				
< 17	0.65	0.0	2.61	< 0.001
17 ≤ age < 25	18.36	10.63	41.74	
25 ≤ age < 35	69.11	76.15	47.83	
≥ 35	12.53	13.22	10.43	
Family status (single)	46.34	42.12	59.13	0.002
Nationality				
French	92.46	96.85	79.13	< 0.001
EU migrants	1.08	0.57	2.61	
Non-EU migrants	6.47	2.58	18.26	
Level of education				
No schooled	0.43	0.29	0.87	< 0.001
Primary/Secondary school	20.91	12.32	46.96	
High school	15.95	12.32	26.96	
Higher education	62.72	75.07	25.22	
Employment status				
Employed	73.32	84.10	40.87	< 0.001
Parental leave	1.08	1.16	0.87	
Unemployed	2.82	1.73	6.09	
Other	22.78	13.01	52.18	

lived single, had lower level of education, and less employed (Tab. I).

More deprived women smoked during pregnancy (p = 0.001). They also had had more frequently voluntary termination of pregnancy (p < 0.001). Prenatal follow-up were poor among the most deprived women who were four fold without quality of prenatal care and two fold without a standard perinatal follow-up (Tab. II).

A significance difference of birth weight has been identified according to the social deprivation with more babies having low birth weight (< 2,500 g) or high birth weight (≥ 4,000 g) in deprived women (Tab. III).

Women's nationality was the sole confounding factor identified (p = 0.011). Individual social deprivation was associated with adverse perinatal outcomes in the bivariate analysis with RR equal to 1.49 (95%CI: [1.01-2.21]). The multivariate analysis endorsed this result with aOR equal to 1.95 (95%CI: [1.15-3.29]) after adjustment on nationality (Tab. IV). The a posteriori power of our study ($\alpha = 0.05$) was 62% (unilateral test).

Tab. II. Descriptive and bivariate analysis of women's medical and obstetric characteristics and of new born medical characteristics in the overall sample and according to the social deprivation status.

	Overall sample % (N)	Non-deprived % (N)	Deprived % (N)	p
Parity (Nulliparous)	39.44 (464)	41.55 (349)	33.04 (115)	0.11
Type of pregnancy (single)	97.63(464)	97.42(349)	98.26(115)	1.00
Pregnancy Tobacco smoke (yes)	17.06 (463)	13.79 (348)	26.96 (115)	0.001
BMI[a] during pregnancy (≥ 25)	22.63 (464)	22.92 (349)	21.74 (115)	0.79
Obstetrical history	(463)	(348)	(115)	
Induced abortion	12.28	9.17	21.74	< 0.001
Miscarriage	14.87	16.33	10.43	0.12
Caesarean section	7.76	7.16	9.57	0.40
Prenatal follow-up	(460)	(348)	(112)	
< 4 prenatal visits	1.30	0.57	3.57	0.03
< 7 prenatal visits	10.43	8.05	17.86	0.003
Preterm birth (< 37 weeks of gestation) (yes)	9.24 (476)	8.10 (358)	12.71 (118)	0.13
High blood pressure during pregnancy (yes)	7.46 (456)	6.45 (341)	10.43 (115)	0.16
Gestational Diabetes (yes)	5.05 (455)	4.69 (341)	6.14 (114)	0.54

[a] BMI: Body Mass Index

Tab. III. Descriptive and bivariate analysis of obstetrical data about labour and delivery and of new born medical characteristics in the overall sample and according to the social deprivation status.

	Overall sample % (N)	Non-deprived % (N)	Deprived % (N)	p
Onset of labour	(474)	(356)	(118)	
Spontaneous labour	63.50	63.76	62.71	0.98
Induction of labour	22.36	22.19	22.88	
Elective caesarean section	14.14	14.04	14.41	
Mode of delivery	(476)	(356)	(118)	
Caesarean sections	21.43	21.79	20.34	0.74
During labour	34.31	35.90	29.17	0.54
Before labour	65.69	64.10	70.83	
PPH[a] (yes)	4.22 (474)	3.63 (358)	6.03 (116)	0.29
Birth weight (grams)	(476)	(358)	(118)	
< 1,500	2.32	2.81	0.85	0.03
1,500 ≤ weight < 2,500	5.91	4.49	10.17	
2,500 ≤ weight < 4,000	86.71	88.48	81.36	
≥ 4,000	5.06	4.21	7.63	
< 5th percentile	4.83	4.75	5.08	0.88
Five-minute Apgar score ≤ 4	1.68 (475)	1.12 (357)	2.54 (118)	0.37

[a] PPH: Post-Partum Haemorrhage.

Tab. IV. Results of the bivariate and multivariate analysis for adverse perinatal outcomes according to the social deprivation status.

	Bivariate analysis			Multivariate analysis	
	Non-deprived % (N)	Deprived % (N)	Crude RR[a] [95%CI[b]]	Adjusted OR[c] [95%CI[b]]	p
Composite criterion	16.48 (358)	24.58 (118)	1.49 [1.01; 2.21]	1.95 [1.15; 3.29]	0.012

[a] RR: Relative Risk; [b] CI: Confident Interval; [c] Adjusted OR: Adjusted Odds Ratio on the nationality (French vs. foreigner)

Discussion

Main results

Women who were socially deprived were exposed to higher risk of adverse perinatal outcomes. They also had poor prenatal follow-up and poor quality of prenatal care.

Comparisons with other studies

Four French studies have already identified association between individual social deprivation and perinatal indicators [19, 20-22]. Sass et al. showed that EPICES score was associated to poor gynaecologic follow-up (adjusted OR 2.09 [2.02; 2.16]) [19]. Gayral-Taminh et al. revealed that socioeconomic and social deprivation measured by an individual questionnaire of 67 items were associated to preterm birth (aOR 1.38 [1.06; 1.79]) and five-minute Apgar score < 7 (aOR 2.98 [1.43; 6.18]) [20]. Convers et al. reported higher prevalence of gestational diabetes, high blood pressure during pregnancy and intrauterine growth restriction in the most deprived women [22]. The last French national perinatal survey performed in 2010 showed poor prenatal follow-up in migrant and in women having low income status (aOR 1.4 [1.1; 1.9]) [21]. Several American and European studies also revealed association between socioeconomic deprivation and adverse perinatal outcomes by measuring deprivation with geographical indices [8-16, 30-35]. Most of the surveys identified a significant relationship between preterm birth and neighbourhood deprivation [10, 12-16, 30, 32-34]. Additional works revealed an association between other adverse perinatal outcome and deprivation as small for gestational age [15], low apgar score at 5 minutes after birth [15], low birth weight [31], perinatal mortality [9, 15, 34], stillbirth [16]. The review of literature also revealed that association existed between adverse perinatal outcomes and deprivation was weighted by pregnant women migration status with misunderstood causal mechanisms as ours findings [11, 15, 35].

Implications

Deprived women had specific characteristics, they were migrants, younger, with less social support, lower level of education, lower employment rate, poorer medical follow-up and riskier behaviour (tobacco smoke espe-

cially). Migrant status need to be consider in particular because it was the only confounding factor identified even though one of the inclusion criteria was fluent command of spoken and written French. It would be interesting therefore to deep analysis in these women known to have limited access to care [36]. This result also underlies that deprived women deserve specific consideration not only by various healthcare professionals like obstetricians, midwives, psychiatrist and general practitioners, but also by social workers. Consequently, family policy that takes into account the whole family, from the beginning of pregnancy to the post-partum period should be implemented in maternity hospitals. Such a program should take in consideration medical and social needs deprived women have and also develop appropriate educational processes.

Various tools exist to measure deprivation based on composite indices per geographic area like Townsend, Carstairs, NZDep index and one index from a North American study [1, 2, 23, 24]. EPICES score is the only one that measure individual deprivation. Our results were congruent with those of the literature, the EPICES score seems to be a reliable tool to measure deprivation in pregnant women [37]. The item on physical activity could be irrelevant in the context of pregnancy. It appeared that it was not the case according with the literature that underlined the importance to maintain regular physical activity during pregnancy [38, 39]. The EPICES score should be therefore included systematically in standard follow-up of pregnant women.

Strength and limits

It was a multicentre population-based study performed on a moderate sample size with high participation rate. The research team decided to not include pregnant women having a voluntary termination of pregnancy considering psychological reasons. Women having termination of pregnancy, 10.6 for 1,000 women aged from 15 to 49 years in Auvergne in 2006 (from the regional observatory of the health of Auvergne; www.ors-auvergne.org), are a specific group that deserve to be investigate apart. There is therefore no selection bias. Our statistical analysis was limited by insufficient statistical power.

Conclusions

Deprived women were at higher risk of poor prenatal follow-up, poor quality of prenatal care and adverse perinatal outcomes. The EPICES score seemed to be a reliable tool to identify deprived pregnant women. Further research is needed therefore to assess adverse perinatal outcomes under the prism of individual social deprivation and to look for barriers that prevent pregnant women to fulfil standard follow-up.

Acknowledgment

We would like to thank the AUDIPOG Sentinel Network (The Association of Users of Computerized Data in Pediatrics, Obstetrics and Gynaecology - Association des Utilisateurs de Dossiers Informatisés en Pédiatrie, Obstétrique et Gynécologie), who took part in this project.

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Appendix 1 EPICES score

(Evaluation de la Précarité et des Inégalités de santé dans les Centres d'Examens de Santé - Evaluation of Deprivation and Inequalities in Health Examination Centres)

Questions	Yes
Do you sometimes meet with a social worker (welfare worker, educator)?	10.06
2. Do you have complementary health insurance (mutual insurance)?	11.83
3. Do you live as a couple?	8.28
4. Are you a homeowner or will you be one in the near future?	8.28
5. Are there periods in the month when you have real financial difficulties in facing you needs (food, rent, electricity)?	14.80
6. Have you participated in any sports activities in the last 12 months?	6.51
7. Have you gone to any shows (cinema, theatre) in the last 12 months?	7.10
8. Have you gone on holiday during the past 12 months?	7.10
9. Have you seen any family members in the past six months (other than your parents or children)?	9.47
10. Did you have difficulties (financial, family or health), is there anyone around you who could take you in for a few days?	9.47
11. Did you have difficulties (financial, family or health), is there anyone around you who could help you financially (material aid such as lending you money)?	7.10
Intercept	75.14

ORIGINAL ARTICLE

Occupational Medicine and Hygiene: applied research in Italy

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Key words

Stress and violence • Biological risk • Sleep hygiene

Summary

The goal of Occupational Medicine and Hygiene is that of ensuring safety, health and well-being at workplaces, mainly assessing and preventing existing occupational risks. Scientific research in this field can provide useful arguments and further evidence upon which effective, efficient and sustainable policies and preventive measures have to be chosen and applied by the occupational physician in work-life. This paper summarizes four original studies, conducted in different professional settings across Italy, focusing on critical items, such as stress and violence, biological risks and sleep hygiene. The knowledge obtained can be useful to orientate proper preventive programs aimed at improving workplace health.

Introduction

Applied research in the field of Occupational Medicine and Hygiene can provide arguments and scientific evidences to properly choose and implement effective, efficient and sustainable policies and preventive measures in order to ensure safety, health and well-being at workplaces. In this way, a strong basis for the development, application and constant monitoring of practical methods and tools can be provided to the occupational physician at the company level, both in the public and private settings.
Occupational Hygiene has a broad scope, in that deals with the protection, safety and health of workers from work-related hazards, systematically anticipating, recognizing, evaluating and controlling the risks, which can be subdivided into biological, chemical, physical, ergonomic and psychosocial ones [1].
In this article, four original researches performed in different workplaces across Italy, focused on some critical issues in the field of occupational safety and health and the main categories of work-related risks (i.e., the assessment and management of the risks associated to stress, violence, and biological exposure in workers employed in health-care settings, together with those associated with sleep disorders and sleepiness among truck drivers), are summarized below.
These studies represent the scientific activity carried out by the National Working Group in Occupational Hygiene born within the Italian Society of Hygiene, Preventive Medicine and Public Health (SItI), with the collaboration of both occupational medicine and hygiene units. The highly interdisciplinary nature of this Working Group is reflected by its effort of integrating the knowledge, the goals and skills of the two disciplines for the sake of safety of the worker in different workplaces.

Study 1: Risk assessment of stress and violence in an Italian regional referral Hospital

Introduction

The work-related stress is a significant aspect in the management of health and safety in healthcare, both because of the peculiarity of the sector and because of the progressive reduction of resources, particularly human ones, resulting in continuous and repeated linear cuts to spending. This type of spending review acts indifferently on both not perfectly efficient areas and on areas with an appropriate allocation of resources, without a prior analysis and reorganization of the processes; the consequences are, often, an unjustified increase in the workload, feeling of inadequacy, and frustration [2, 3].
The actual legislation requires the employer to evaluate all risks for health and safety, including those caused by work-related stress, and refers to the indications of the

Standing Advisory Commission on health and safety at work for the details of such assessment (Law Decree 81/2008, art.28, comma 1-bis) [4, 5].
The Commission has identified the criteria to be considered: sentinel events (accident rates, sick leave, turnover, reports of physicians); job characteristics (environments and workloads, working hours and shifts, limited knowledge of the procedures and/or inadequate skill); and correspondence between worker skills and job requirements [6].
A special role is played by the episodes of aggression towards workers, both verbal and physical, increased in the last years [7]. Occupational or work-related violence in health-care settings is a growing phenomenon that can be defined as an incident in which a perpetrator abuses, threatens, or assaults a health-care worker (HCW) in work-related circumstances. Gillespie and collaborators have reviewed the main HCW risk factors: these are gender, age, years of experience, workload, relationship status, and previous workplace violence training [8].

Methods

The IRCCS AOU San Martino-IST of Genoa, Italy, the Regional teaching and research hospital of Liguria Region, established an internal working group to assess the work-related stress and the specific risk factors represented by attacks on workers. The group consists of the Occupational Medicine Unit, the Prevention and Protection Service, the Clinical Psychology Unit, the Psychiatric Unit, the Workers' Representatives for safety (RLS), and the various components of the Executive Board. In the absence of a National or Regional benchmark, the Working Group decided to stratify the risk levels within the IRCCS, in order to obtain a priority scale of remedial measures.
The group elaborated a set of indicators divided into three areas:

- risk indicators: hours worked, missed holidays, overtime, night and holidays shifts, tasks breaks, characteristics of the environments, complex postures and positions, medical restriction, adverse events and complaints, performance during emergencies, critical patients, etc.;
- disclosure indicators: days of absence, number of accidents, questions of internal mobility and transfers, appeals to the reception, occupational stress, etc.;
- contrast indicators: internal communication system and methods for the reception of new employees, training, internal audits.

The indicators were measured at the level of the single operating units and normalized by the number of workers; then, for each one, the average or median, depending on their distribution, was computed. For each operating unit, the indicator was considered expression of the risk if placed in the negative side respecting to the central position index; the percentage of negative indicators out of the total of the available indicators provided a measure of the level of work-related stress in the operative unit, considering the IRCCS as benchmark.

Regarding the attacks on workers, a preliminary analysis of some operating units (medicine, neurology, emergency room, intensive care, infectious diseases, and psychiatry) was carried out through the Overt Aggression Scale (OAS) questionnaire filled in by the operators over a period of four months. The questionnaire, which allows the description of the various aspects of each episode of violence, has been entered in a database and subjected to statistical analysis using multidimensional techniques, like Correspondence analysis, which enables to display categorical data in two-dimensional graphical form.

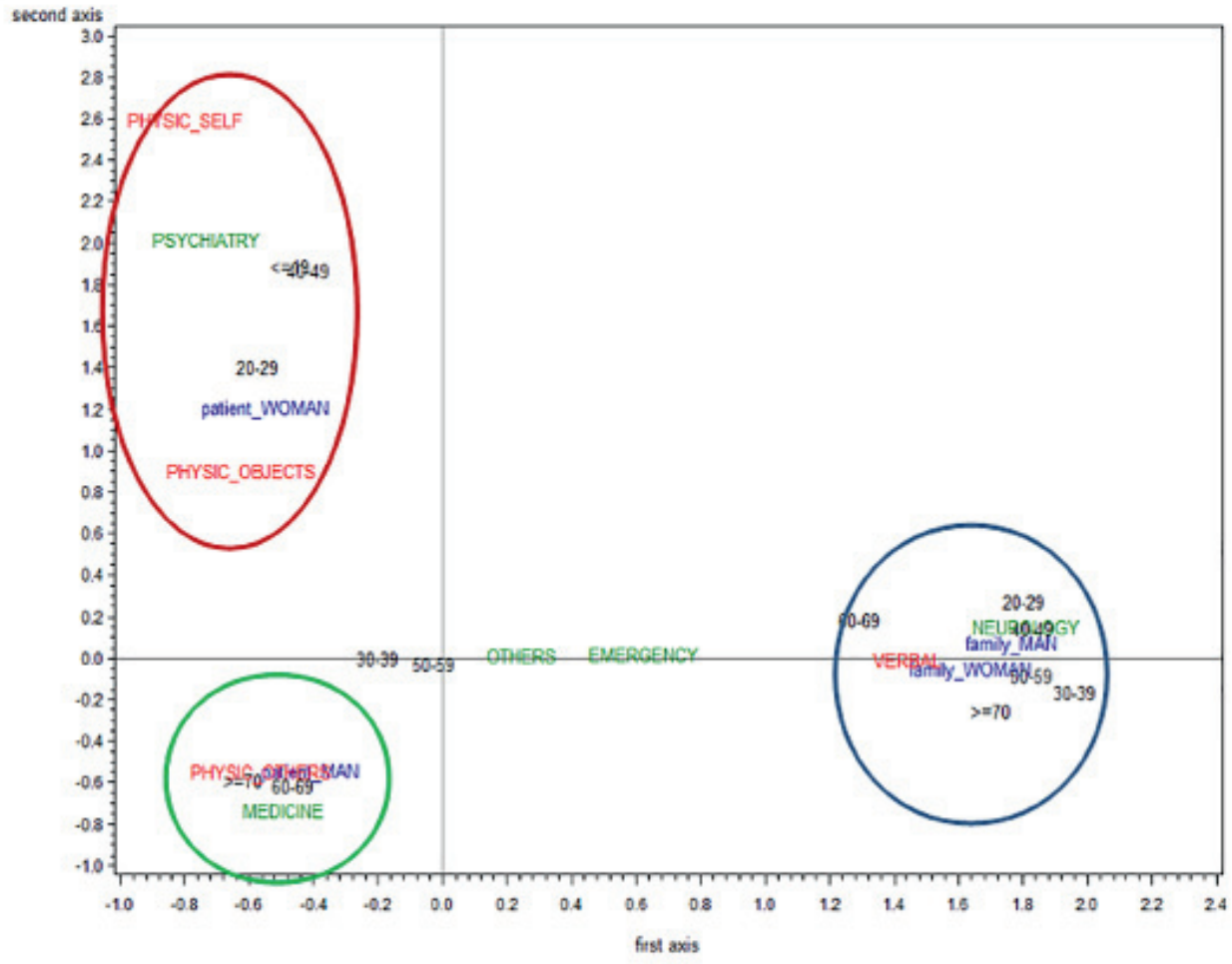
Results

The stress evaluation with the method described above during three years allowed us to identify the areas at greatest risk inside the IRCCS (namely, medicine, neurology, emergency room, intensive care, infectious diseases, and psychiatry) on which work was carried out through focus groups and further investigation by the operative unit of Clinical Psychology. In addition, the table of risk stratification has facilitated the redeployment of workers to which the Occupational Medicine Unit ordered exposure restriction to this specific factor, for pre-existing pathological conditions or individual hypersensitivity (insufficient coping strategies).
The recording of OAS questionnaires on violence has enabled the analysis of 267 episodes, of which approximately 50% with physical aggression (towards the operators, but also objects and oneself). The distribution of their characteristics: aggressor (patient, family or other persons), kind of event (physical aggression preceded or not by verbal aggression) and the victim (the operator, the objects, the aggressor himself, in various combinations) was significantly different depending on the type of departments. The various features were also associated with each other in an absolutely not random way; in the departments of medicine, there were mainly aggression by elderly male patients, while in the departments of neurology, patient's relatives of both sex verbally assaulted staff; in psychiatry unit, it was mainly the young female patient to implement violence mostly to herself and to the objects (Fig. 1). Even the temporal distribution during the day differed significantly in various types of departments. In particular, peaks in early morning, afternoon/early evening could be noticed in psychiatry and emergency room departments, respectively. No peaks could be detected in neurology ward, whilst distribution in medicine wards was varied throughout the day.

Conclusions

The evaluation of work-related stress made in a structured way, through indicators and specific questionnaires, is a valuable tool to identify areas at risk and their characteristics. It enables the development of preventive measures and identifies their key-objectives as well as facilitates the management of workers with limitations.

Fig. 1. Correspondence analysis. Distribution of event characteristics: type and age of the aggressor, type of the aggression, setting of the aggression (hospital unit).



Notes: Data collected with Overt Aggression Scale (OAS) questionnaire; statistical analysis performed with SAS procedures rel.9.2

Study 2: Sleep disorders and sleepiness: major risk factors in road accidents and injuries for truck drivers

Introduction

Excessive daytime sleepiness (EDS) is a common condition in our society that can be caused by both physiological and pathological factors. The physiological origin of EDS comes from a modern irregular lifestyle and is due to the rhythms of our "24 hours society" and work organization (shift work, night work, extratime, overtime), that causes sleep debt and the disruption of the circadian sleep-wake rhythm. The pathological causes of EDS are various but the main one is Obstructive Sleep Apnea Syndrome (OSAS). OSAS is a chronic breathing disorder, characterized by recurrent episodes of partial or complete obstruction (hypopnea / apnea) of the upper respiratory airways during sleep, that lead to a decrease in nocturnal oxygen saturation and micro-awakenings. Accordingly, OSAS causes sleep fragmentation, non-restorative sleep and EDS; moreover, it is the main risk factor for serious diseases, such as metabolic disorders, diabetes and cardio/cerebra-vascular disorders [9].

Studies conducted over the past twenty years show a significant relationship between sleep disorders, EDS and accidents/injuries [10]. EDS is indeed the cause of more than 20% of all traffic accidents [11] and increases the risk of being the victim of a fatal accident eightfold [10]. Truck drivers are considered the group of workers most exposed to the risk of EDS-related accidents. This is mainly due to the high prevalence of OSAS among professional drivers [12], the work rhythms and the sleep habits of these workers. The aim of this study, done for the first time in Europe in a mobile medical clinic equipped with advanced technology, was to evaluate the prevalence of sleep debt, OSAS, EDS and traffic accidents in a sample of truck drivers.

Methods

This study was carried out as part of "CNH Iveco Industrial Check-Stop Project", an international program of

prevention and information for road safety in collaboration with the Departments of Neuroscience and Health Sciences of the University of Genoa, and sponsored by the Ministry of Transport, the *Unione Interporti Riuniti* and the Italian Association of Sleep Medicine (AIMS).
The research was carried out in 2014, in the major Italian trucking hubs (Turin, Novara, Verona, Bologna, Rome and Naples). The clinical protocol included different phases:

a) standardized and validated questionnaires (Epworth Sleepiness Scale - ESS; Berlin Questionnaire) and a structured interview about sleep habits, sleep disorders (sleep disorders score - SDS) and driving safety and accidents that have occurred in the past three years;
b) semeiotic and clinical evaluation, and screening for sleep disorders and EDS;
c) identification of potential subjects with suspected OSAS and / or EDS;
d) information / education for each participant who resulted negative in the sleep disorder and EDS screening, regarding sleep hygiene and healthy lifestyle behaviors for the prevention of accidents and EDS-related injuries.

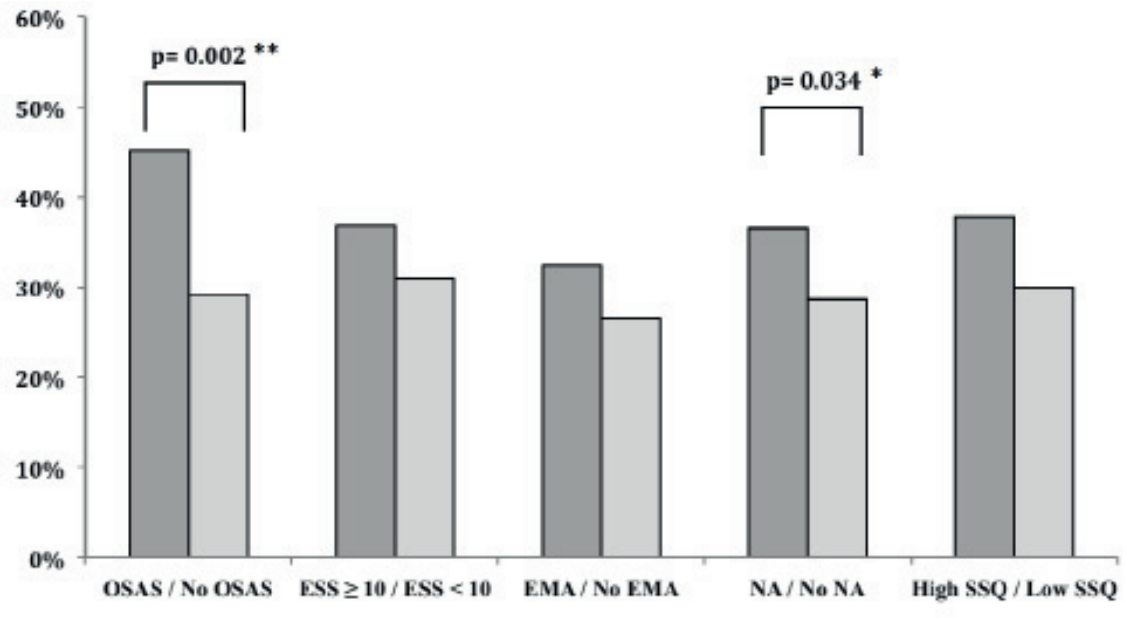
Results

The data presented in this study are preliminary and based on a sample composed of 728 drivers. The participants were all males, from across the country but belonging to different ethnic groups, with a range of age between 18 to 81 (mean 44.2 ± 10.2 years) and a BMI (body mass index) average of 28.5. The presence of at least one co-morbidity, such as hypertension, dyslipidemia and diabetes, was found in 36.3% of participants.
A condition of sleep debt because of nighttime awakenings or early morning awakenings was found in 21.3% of the analyzed sample. Only 31.4% of participants were satisfied with the quality of their sleep. EDS was reported in 10% and for 37.2% was expressed a suspicion of OSAS; of these, 57% had at least one co-morbidity, such as hypertension, dyslipidemia and diabetes. 31.5% had at least one accident / injury in the last 3 years. Figure 2 shows the association between OSAS, sleep debt, EDS and traffic accidents.

Conclusions

The preliminary data obtained confirm the high prevalence of sleep debt and OSAS in truck drivers reported in the literature. From the results emerges an important relationship between sleep debt and the presence of traffic accidents [13]. These results confirm the urgent need for a prior assessment focused on EDS and OSAS in the activities of primary prevention and health surveillance [14] especially in the categories of workers that require high levels of alertness and therefore at greater risk of accidents/injuries. It is important to manage the health risks and safety of professional drivers by minimizing risk factors inducing EDS through screening of sleep disorders together with other lifestyle approaches.

Fig. 2. Accidents at workplace in participant with and without OSAS, EDS, sleep debt, and poor subjective sleep quality.



Notes: OSAS: Obstructive Sleep Apnea Syndrome; EDS: Excessive Daytime Sleepiness assessed with Epworth Sleepiness Scale (ESS); EMA: Early Morning Awakening; NA: Night Awakening; SSQ: Subjective Sleep Quality. *statistically significant, with a p-value < 0.05; **statistically significant, with a p-value < 0.01.

Study 3: Assessment of the compliance with hygiene and safety procedures, and their perception by professionals, within a network of dental practices in Rome, Italy

Introduction

A number of studies has been published regarding the critical issues on the health and safety at workplace within dental practices. In recent years, notwithstanding the technical progress made, problems, including injuries by percutaneous exposure, exposure to bio-aerosols, radiations, chemical agents and noise, persist in this setting [15]. Other professional items, such as muscular-skeletal disorders, dermatitis, eye and psychological problems, have also been notified [16]. Strategies aimed at reducing the number of unfavourable events include on-going education, hygiene of the premises, personal protection equipments, adequate sterilization and other high-level disinfection techniques. Some studies, mainly based upon self-administered questionnaires, have investigated procedures, educational programmes, hygiene conditions and the complications associated to HBV, HCV and HIV infections reported among dentists in various Regions and cities in Italy. Table I summarizes these studies searched in a systematic way, looking up for a proper string of key-words such as "hygiene", "dentist" and "questionnaire", using Medical Sub-Headings (MeSH) terms where adequate [17-22]. In a dental working environment a number of procedures can generate bacterial aerosol and droplets and concentrations appear to be higher during interventions involving ultrasonic ablation, or during procedures using high speed drills: it is therefore possible that airborne infectious agents may be transmitted to staff and patients, especially if the overall hygiene procedures in the work-place are not adequate [23]. Some procedures, such as pre-procedural rinsing performed by patients with mouthwash and electrostatic extraction of aerosol during dental procedures, certainly represent useful preventive measures. Further, adherence of the professionals to recommended vaccinations and the proper use of personal protective equipment can reduce the contact with bacterial droplets and aerosols, particularly during invasive and prolonged interventions.

Methods

This report deals with the assessment of the compliance with hygiene and safety procedures, and their perception by professionals, within a network of dental practices in Rome, as found during 'expert' inspections undertaken by the Health Surveillance Authority (SISP-ASL). As part of this investigation, 100 questionnaires, containing 37 multiple-choice questions, were used and completed by dentists belonging to 76 different dental practices.

Results

The sample was mainly composed of males (78% of subjects), professionals aged 50 years or above (59%); 50% of the doctors participating in the study had a degree in dentistry (many also had a further specific qualification), 30% had a degree in medicine and surgery, and/or a second degree in dentistry and/or a special qualification in dentistry, 17% had a degree in medicine and surgery together with other specific courses in dentistry, and, finally, 3% did not provide an answer.

Having a degree or having "work experience" (defined as less than and over 20 years of professional activity) do not appear to influence the perception of the risk of infection due to HBV and HIV: the percentages of those who answered stating that the risk was "high only for surgery manoeuvres" resulted 27% and 41% for the two pathogens, respectively. Preliminary analyses of the answers of the questionnaires highlighted a trend: the higher the work experience was, the lower the percentage of those who underwent vaccination or performed periodical check-ups for HBV infection was recorded. Furthermore, it appears that knowledge is insufficient among professionals (28-32%) regarding recent regulations aimed at preventing injuries from needles and sharp objects (Law Decree 19.2.2014 n.189 which modifies Law Decree 81/2008): 62% of the sample stated to always put the cap back on the needles and 2% re-caps them occasionally.

With respect to the knowledge about the prevention of blood-borne viral infections, 46% of the study sample believed that previous information on the HIV-seropositive status of the patient was always indispensable, while 18% declared the same only when the assistance was given to high-risk subjects (i.e., intravenous drug users, chronic patients and carriers). Behaviours regarding prevention and safety in this field could be improved also with respect to the proper use of goggles, worn only in 60% of the dental treatments.

With respect to professional training and education, more than 95% of the dentists stated that the source of knowledge was represented by academic or scientific sources or by scientific congresses and meetings.

Analytical control of the water system was carried out only in nearly half of the dental practices surveyed, even if 90% of the interviewed stated they used some filtration system at the workplace. Tweaks and works were undertaken mainly with resins and aspiration in 54% of the treatments; a microbiological assessment of the work-environment contamination inside the studio was performed in 32% of the dental practices. The formal assignment and the real activity of an Occupational physician resulted to occur in 67% of the dental studios.

By applying Regional check-lists regarding the adequacy of the furnishings and work environments, it was found that the environments (cure / acceptance / waiting room) were perfectly separated in 65% of the practices, while proximity between administrative and medical spaces was found in 35%. The presence of expired pharmaceuticals resulted in 15% of the practices and the regular stocking of waste materials was performed in 60% of

Tab. I. Summary of the studies, undertaken by questionnaires, investigating the safety and the hygiene conditions in dental practices across Italy.

Parameters	M.G. Galli et al., 2000	C. Germinario et al., 2001	M.T. Montagna et al., 2003	L. Veronesi et al., 2004	F. Vitale et al., 2005.	Fabiani et al., 2006	M.L. Cristina et al., 2009
STUDY CONTEXT Dental studios	Public and privates in Milan	Privates in Bari	Privates in 11 Italian cities	Privates in Parma	Privates in Palermo	Privates in L'Aquila	Private and public sector in Genoa
OBJECTIVES Evaluation of	Awareness of risk factors of different nature (chemical, physical, biological)	Awareness and practice for prevention of infection	Awareness of infection risks and control procedures	Awareness and measures adopted within procedures of infection control	Awareness, aptitude and measures adopted for control of infections and programming of awareness and educational intervention	Awareness and measures adopted within procedures of infection control	Procedures and measures adopted for control and prevention of infections
MATERIALS & METHODS	Anonymous questionnaire to be returned + telephone survey	Anonymous questionnaire to complete and return	Anonymous questionnaire to complete and return	Questionnaire sent by email	Anonymous questionnaire to complete and return	Validated questionnaires to complete and return	Questionnaire by interview or sent by post
SUBJECTS RECRUITED Responders/ Total (%)	116/231(50.2%)	94/200 (47.0%)	444/1000 (44.4%)	122/400 (30.5%)	254/675 (37.6%)	82/127 (64.6%)	106/176 (60.2%)
PERSONAL DATA RESPONDERS							
Average age (±DS)	40 years (range 25-68)	40 years	42.2 years (DS=9.4)	43years (DS=9.7)	43.6 years (±DS=9.7)	41 years (DS=8)	< 30 years in 10.4% of cases, 30-50 years in 73.6% of cases, >50 years in 16% of cases
- Degree	47% with a degree in Medicine and a specialization in Stomatology, 28% with a degree in Medicine, 25% with a degree in Dentistry	76.6% degree in Medicine	60.4% degree in Medicine	62% degree in Medicine	47.7% degree in Medicine	53% degree in Medicine, 34.9% degree in Dentistry, 47% in odontology and dental prosthesis	NA
- Work experience (average years ± DS)	1-42 years	13.3 years	14.2 years	15 years	14.5 years	13 years (DS = 7)	NA
- Hours worked (average hours/ week)	< 20 hours/week in 10% of cases, 20-29 hours/week in 11% of cases, 30-39 hours/week in 25% of cases, 40-49 hours/week in 38% of cases, > 50 hours/week in 16% of cases	27.8 hours/week	20-49 hours in 69.2%	20-49 hours	30-50 hours in 57.7%	13.5% about 20 hours/ week, 78% 20-49 hours/ week, 8.5% > 50 hours/ week	NA
Number of patients/day (average)	NA	NA	NA	NA	NA	NA	< 10 patients/day in 26.4% of cases, 10-20 patients/day in 36.8% of cases, >20 patients/ day in 36.8% of cases
BIOLOGICAL RISK: KNOWLEDGE, EVALUATION (Focus on)	Epidemiological (transmission and exposure, comparative risk evaluation)	Epidemiological (transmission and exposure, comparative risk evaluation)	Epidemiological (exposure, risk categories)	Risk perception and knowledge	Epidemiological (transmission, biological samples, categories at risk, comparative risk evaluation)	Epidemiological (exposure, risk categories)	Risk awareness
VACCINE	76% vaccinated for HBV, seroconversion verified in 72% of subjects Diagnostic checks for HBV (72%), HCV (70%), HIV (70%)	75% HBV and 53% has immunized staff	79.5% HBV, only 55.2% verified seroconversion Diagnostic checks for HBV (67.8%), HCV(67.8%) HIV (57,6%)	89% HBV and 72% seroconversion	76.2% believe HBV necessary	82% HBV and 70% seroconversion Diagnostic checks: for HBV (76.5%) for HCV (78%) and for HIV (69.5%)	86.8% HBV
PROFESSIONAL INJURIES / ILLNESSES	Accidental exposure to hazards (20% wound by needles at least once in last years, 13% at least twice in last years, 8% at least 3 times) Professional illness (5 cases of HBV, 2 cases of HSV)	Accidental injuries with needles or sharp (63% yes, sometimes and 1% frequent)	Accidental (38.5% with needles) Infective disease (1,9% hepatitis)	Accidental injuries with needles or other within last 5 years (73% yes) Professional illness (HBV 1.6%, HSV1 0.8%)	Pricks and cuts in last year (43.1% rarely / often)	NA	NA

the cases. A surface with windows or the aeration unit with adequate air replacement was identified in 65% of the cases, whilst automated washbasins were not present in 80% of the studios. Class B autoclave was missing in 2% of the studios, and instrumental non-compliance of the studios and an insufficient quality of clinical records and the risk assessment document were identified in 2% of the dental practices, with more than 25% of these shortcomings regarding emergency and maintenance procedures.

Various cases highlighted the advantageous and adequate use of high-technologies such as thermal destruction of needles used or the reclaiming of water through disinfection; flushing procedures were adopted in many cases. A lack in the proper management of physical risk factors (i.e., artificial optical radiation, hand arm vibrations, work-related musculoskeletal disorders - WMSDs for use of cutters and drills) was generally observed.

Conclusions

The results of the investigations suggest that further efforts are required in dental practices regarding both the assessment and the management of some occupational risks (i.e., biological exposure to blood-borne and airborne infectious agents, environmental issues and technical procedures) and the compliance of professionals

with the existing recommendations and laws aimed at preventing exposure to different hazards. Although the standards of the working environment appeared generally acceptable in this work-place, another critical issue is represented by the fact that not all the needed safety procedures are always available, formalized and routinely updated and verified in the risk assessment document of the company.

Study 4: Risk assessment of Health-care Associated Infections among Health-Care Workers in a Local Health Authority in Sardinia Region, Italy

Introduction

Health care workers (HCWs) are more prone than other workers to biohazard related to potentially infectious biological agents. Transmission in the health-care settings can occur through several ways, mainly blood-borne and airborne/droplets, and it can affect both HCWs and patients [24].

In the last twenty years, scientific organizations and authors set several alerts on the need to proper risk assessment and management of biohazard in the health-care sector [25, 26]. In spite of these alerts, some critical issues still remain unsolved in hospitals and other health services.

Objective of the study was to assess the risk of Health-care Associated Infections (HAIs) in HCWs of a Sardinian Local Health Authority, in order to identify proper and tailored preventive interventions to control this existing risk.

Methods

A risk assessment campaign was conducted from May to October 2014 according to the recommendations of the Italian Society of Occupational Health and Industrial Hygiene (SIMLII) [27].

We considered as HCWs all employees working in healthcare settings with potential exposure to patients and/or to infectious materials, including physicians, nurses, nursing assistants, therapists, technicians, emergency medical personnel, pharmacists, laboratory personnel. The blood-borne risk assessment entails data collection on: (1) identification of the infectious agents at the workplace; (2) identification of the source and description of the pattern of transmission; (3) surveillance of the infections among HCWs; (4) surveillance of the accidental exposures; (5) characteristics of the work environment, medical devices, personal protective equipment, organization of the preventive system.

The identification of the infectious agents has been made through consultation of scientific evidences, national and local epidemiological data. We collected transmission modalities through the analysis of exposure procedures performed in each unit (stratified by job task). Data on immunization coverage among HCWs, presence of infectious diseases and injuries were collected from the database of the Occupational Physician, whereas data on protective equipment and disposals were collected during inspections in the units. Exposure procedures and injuries were collected following the recommendations provided by the CDC of Atlanta and the Society for Healthcare Epidemiology of America [27, 28].

Risk assessment for tuberculosis (TB) was limited only to three hospitals, due to the absence of data from the country health services. Number of beds in the structure and notifications of TB cases in the period 2010-2014 were considered, following the algorithm of the guidelines proposed by the CDC [29]. The TB infection risk was then stratified per year and unit following the indications from the Italian Ministry of Health.

Data on seasonal influenza vaccination coverage and characteristics of risk communication adopted by health management were collected.

Seroprevalence ratios of antibodies against measles, mumps, rubella and varicella among HCWs were estimated from the medical records of the health surveillance service. Data on outbreaks in the community and in hospitals in the last three years were collected from the Local Public Health Unit.

The presence of procedures for scabies, *Neisseria meningitidis* and *Ebola* and their adherence to international scientific recommendations were also assessed.

Results

Overall, 2,661 HCWs were enrolled in the study. Blood-borne biological agents considered were HBV, HCV, HIV and *Ebola*. Exposure prone procedures were more frequently performed by medical doctors in surgery, emergency and intensive care units, whereas procedure considered at lower risk were more frequently performed by nursing staff in all clinical units. The seroprevalence of blood-borne pathogens among HCWs was < 1% for HBV, < 2% for HCV, and > 0.1% for HIV. Among blood-borne-positive HCWs, there were not specific clusters by job task and unit. Seroprotection for HBV was lower than 70%. Blood-borne injuries represented the most common cases (2011-2013: 130/283; 45%). The majority of them were percutaneous (n = 116; 89%) and occurred during intravenous injections and recapping or disposing of needles (41%), while 7% during exposure prone procedures. Nurses were more exposed to risk of blood-borne injuries, especially in emergency and intensive care units. Personal protective equipments were widespread in all units, whereas medical and surgical disposal with protective devices were not. In some units these disposals were present but not fit to medical and surgical procedures performed. The system of reporting and follow up of injuries proved to be effective, but not all HCWs were aware of all aspects of the post exposure procedures (in particular with respect to the correct timing of reporting). Procedures for the management of *Ebola* cases were available and coherent with the guidelines of the Italian Ministry of Health. No patients with diagnosed or suspected *Ebola* were ever hospitalized in the authority.

The risk of TB resulted high in one of the three hospitals with a mean of 3.2 TB cases (range 2-4 in 2010-10/2014) per year. Specific preventive procedures and collective protective devices to reduce TB risk were not systematically applied. Emergency and radio-diagnostic units resulted to be at highest risk due to the frequency of contacts with patients with active TB and the lack of adherence to preventive measures compared with other units. In that hospital, in the last two years, three HCWs resulted positive at the *in vitro* test after a contact with a TB patient: protective procedures were not completely applied in all patients with TB.
The influenza vaccination campaign was conducted by communication to units and nurses managers and resulted in vaccination coverage rate less than 10% in the 2013-2014 season.
Overall about the 50% of total HCWs was tested for exanthematic diseases. The sero-prevalence ratios were lower than 95% for measles (1105/1163) and varicella (1100/1159), 84% for mumps (996/1179) and 90% for rubella (1115/1241), resulting in more than 400 susceptible HCWs for at least one of the four viruses. About 100 seronegative HCWs were employed in units with higher risk of transmission (i.e. pediatric and emergency units) and in those with higher risk for patients (oncological, cardiological and nephrology and dialysis units). In 2014, an outbreak of measles occurred in one hospital with four HCWs infected. Procedures for *Neisseria meningitidis* and scabies were not available.

Conclusions

Risk assessment of biohazard in HCWs represents one of the main issues for occupational health professionals in the healthcare sector. The SIMLII recommendations did not allow to establish an overall risk levels for all biological agents considered, however it was useful to identify specific areas of preventive intervention in order to control the risk. In particular, results highlight the need of intervention for training nurses and nursing aides in specific units, focused on both high and low risk procedures, the possible under-reporting of biological injuries of medical HCWs during high-risk procedures, lack of diffusion of medical and surgical disposal with protective devices, inadequate HBV, influenza, and exanthematic disease seroprotection rates, lack of adherence with protective procedures in case of TB exposure, lack of data on TB exposure in country services, and procedures to contain the risk of scabies and *Neisseria meningitidis* transmission. Further efforts have to be done in order to properly manage biohazard in the health-care settings, in order to optimize protection of both HCWs and patients' safety and health.

Conclusions and future perspectives

This article is the summary deriving from the application of different research approaches, conducted in different professional settings across Italy, aimed at improving the knowledge of the specific risks in the workplace, as a necessary step towards their proper management, according to guidelines and standardized procedures. The knowledge obtained can be useful to orientate proper preventive programs aimed at improving workplace health. Implementation of evidence-based sanitary surveillance programs is mandatory for the promotion and protection of workers in this setting.

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