Diplomarbeit

Comparison of clinical and laboratory presentation between PCR confirmed H1N1 influenza infection, rapid diagnostic test confirmed dengue infection and blood smear confirmed plasmodium infection

eingereicht von

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23.09.1986

zur Erlangung des akademischen Grades

Doktorin der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an der

Sektion Infektiologie, Klinische Abteilung für Lungenkrankheiten,

Universitätsklinik für Innere Medizin,

Medizinische Universität Graz

unter der Anleitung von

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Ort, Datum (Unterschrift)

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Danksagungen

Ich möchte mich hiermit recht herzlich bei Herrn OA Dr. med. Holger Flick für die gute Betreuung und Zusammenarbeit bedanken. Er hat mich seit Beginn der Datenerhebung bis zur Fertigstellung dieser Arbeit begleitet und unterstützt. Ohne seine Ratschläge und Ideen wäre diese Arbeit nicht möglich gewesen.

Zugleich möchte ich mich hiermit auch besonders bei Herrn a.o.Univ.-Prof. Dr. Robert Krause für seine Unterstützung bedanken.

Zudem möchte ich mich auch bei meinen Eltern, meinem Freund, meinen Geschwistern und meiner ganzen Familie sowie meinen Freunden aus tiefstem Herzen bedanken, denen ich viel an Geduld und Verständnis abverlangt habe und die mich in den letzten Monaten stets unterstützt, an mich geglaubt sowie die letzten sechs Jahre begleitet haben.

Dankeschön!

Eidesstattliche Erklärung

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Graz, am *Unterschrift*

Table of Contents

Abbreviations

ACT	Artemisinin-based combination therapy
ADE	Antibody-dependent enhancement
ALT	Alanine transaminase
AST	Aspartate transaminase
BPM	Beats per minute
CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
DEET	Diethyltoluamide
DENV	Dengue virus
DF	Dengue Fever
DHF	Dengue Hemorrhagic Fever
DSS	Dengue Shock Syndrome
ELISA	Enzyme-linked immunosorbent assay
ER	Emergency Room
GGT	Gamma-glutamyl transpeptidase, Gamma GT
GISN	Global Influenza Surveillance Network
ICU	Intensive Care Unit
IQR	Inter Quartile Range
JP	Japanese Encephalitis
LDH	Lactate dehydrogenase
LKH	Landeskrankenhaus = state hospital
NS1	ELISA test for detection of circulating dengue nonstructural protein 1
PCR	Polymerase chain reaction
RBC	Red blood corpuscles
RDT	Rapid diagnostic test
RT-PCR	reverse transcription-polymerase chain reaction
TBE	Tick-Borne Encephalitis
WBC	White Blood Cells = Leukocytes
WHO	World Health Organization
WNE	West Nile Encephalitis
YF	Yellow Fever

1 Abstract

1.1 Aim

Returning Travelers frequently present acute febrile illnesses in emergency departments or infectious disease clinics. In this setting, common and important differential diagnoses are dengue-, influenza- and plasmodium infections. Due to the relative low sensitivity and specificity of dengue rapid diagnostic- and influenza tests in the early phase of the disease, it is sometimes difficult to confirm the diagnosis within the first days after the onset of symptoms. Only with the presence of confirmed PCR and dengue rapid diagnostic tests, is the diagnosis of dengue and influenza possible. These tests are often only available when symptoms subside.

The aim of this study is to determine clinical and laboratory differences between dengue and H1N1 influenza infection. The benefit of this study would be, that patients could be treated early (e.g. with neuraminidase inhibitors) when symptoms first appear and preventive measures could be implemented at time.

1.2 Methods

We reviewed 761 patients with PCR confirmed H1N1 influenza infection, RDT (rapid diagnostic test) confirmed dengue infection or blood smear confirmed plasmodium infection from the 1st of January 2005 to the 1st of June 2013. H1N1 influenza PCR was performed at the Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz or at the Institute of Hospital Hygiene, University Hospital of Graz. Dengue rapid diagnostic tests were performed at the Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz or at the Institute of Hospital Hygiene or at the Department of Virology, Medical University of Vienna. Malaria blood smear was performed at the local laboratories. Data was evaluated from the electronic Styrian hospital network, MEDOCS. Participating hospitals were the Medical University Hospital of Graz and the state hospitals of Graz West, Leoben, Bruck, Feldbach, Rottenmann, Bad Radkersburg, Hartberg, Hörgas-Enzenbach, Voitsberg and Judenburg/Knittelfeld. Data sets were available from 199/624 (32%) of H1N1 infected patients, from 49/58 (84%) of malaria infected patients and from 39/79

(49%) of dengue infected patients. Data from the remaining 474 (62%) patients was not available in the MEDOCS database. We compared patients with H1N1 influenza, dengue and malaria with regard to clinical presentation, laboratory parameters and preexisting underlying conditions.

1.3 Results

The median age of the H1N1 group was 16 years compared to 33 years in the dengue and 39 years in the malaria group (p<0,001). 27% of H1N1 patients and 26% of dengue patients presented to emergency room only (and were discharged for further ambulatory care) compared to 6% of malaria patients, whereas 90% of malaria patients were hospitalized and admitted to normal wards compared to 74% of dengue and 66% of H1N1 patients (p=0,007). The median time between the onset of disease and presentation at ER was 1 (IQR 0-2) day in the H1N1 group versus 4 (IQR 1-5) days in the dengue and 4 (IQR 2-4) in the malaria group (p<0,001). A sudden onset was found in only 82% of H1N1 patients, in 95% of dengue patients and in 96% of malaria patients (p<0,015). Fever occurred in 93% of H1N1 patients, as well as in 93% of dengue patients and in 81% of malaria patients (p=0,038).

H1N1 patients were more likely to present respiratory symptoms and signs like cough (73% versus 15% in dengue and 10% in malaria; p<0,0001), rhinitis (24% versus none of either dengue or malaria patients; p<0,0001), wheezing (14% versus none of either dengue or malaria patients, p<0,001), dyspnea (22% versus 4% of malaria patients and none of dengue patients; p<0,0001), thoracic pain (9% versus 4% of malaria and none of dengue patients), rales (10% versus none of either dengue or malaria patients), sore throat (12% versus 8% of dengue and 2% of malaria patients) and pulmonary infiltrates in chest X-ray (19% versus 5% of dengue and none of malaria patients).

Dengue patients were more likely to present with rash (36% compared to 3% in H1N1 and none in malaria; p<0,0001), myalgia (49% versus 24% in H1N1 and 18% in malaria; p=0,004), diarrhea (44% versus 11% of H1N1 and 10% of malaria patients; p<0,0001), headache (67% versus 33% of H1N1 and 39% of malaria patients; p<0,0001) and abdominal pain (23% versus 6% of H1N1 and 6% of malaria patients; p=0,008).

No significant difference was observed in terms of nausea/ vomiting (25% of H1N1, 26% of dengue and 37% of malaria patients) and conjunctivitis (4% of H1N1, 3% of dengue and 4% of malaria patients).

Regarding underlying diseases, only asthma bronchiale (10% of H1N1 versus 2% of malaria and none of the dengue patients; p=0,026) and neurologic diseases (10% of H1N1 versus none of either dengue and malaria patients; p=0,009) were significantly different and more frequent in the influenza group.

In respect to laboratory values, a significant difference was observed in the following parameters: leukocyte level of 6,74 G/l (IQR 5-8,61) in the H1N1 group compared to 5,11 G/l (IQR 3,06-7,46) in the dengue group and 5,86 G/l (IQR 4,52-6,94) in the malaria group (*p=0,002*); thrombocyte concentration of 92 G/l (IQR 65,00-115) in the malaria group compared to 204 G/l (IQR 166-243) in the H1N1 group and 172 G/l (IQR 118-229) in the dengue group *(p<0,0001)*; CRP level of 98,7 mg/l (IQR 55,95-152,5) in the malaria group compared to 17,4 mg/l (IQR 5,5-43,5) in the H1N1 group and 13 mg/l (IQR 3-36,2) in the dengue group (*p<0,0001*); bilirubin level of 1,24 mg/dl (IQR 0,93-2,34) in the malaria compared to 0,4 mg/dl (IQR 0,29-0,67) in the H1N1 group and to 0,43 mg/dl (IQR 0,36-0,62) in the dengue group (p<0,0001); Gamma GT level of 72 U/l (IQR 34-140) in the malaria group compared 25 U/l (IQR 15-49) in the H1N1 group and 46,5 U/l (IQR 27-84) in the dengue group *(p<0,0001)*; AST level of 29 U/l (IQR 24-43) in the H1N1 group compared to 48,5 U/l (IQR 30-87) in the dengue group and 44 U/l (IQR 33-63) in the malaria group (p<0,0001); ALT level of 20 U/l (IQR 15-34) in the H1N1 group in contrast to 43,5 U/L (IQR 28-93) in the dengue group and 44 U/l (IQR 25-70) in the malaria group (*p<0,0001*); and LDH level of 305,5 U/l (IQR 243-414) in the malaria group compared to 223 U/l (IQR 178-270) in the H1N1 group and 267 U/l (IQR 204-394) in the dengue group (*p<0,0001*).

1.4 Summary

Sudden onset of disease seems to appear as frequently in malaria and dengue infections (96% and 95%) as in influenza diseases and is therefore not a useful criterion for differentiation between these diseases. Respiratory symptoms such as cough, rhinitis, wheezing, dyspnea and thoracic pain are much more likely to occur in H1N1 patients and are rarely observed in dengue and malaria patients. Diarrhea

and abdominal pain as well as abdominal findings in physical examination seem to be relatively specific for dengue infections.

Leukopenia occurs in all three groups and is not a useful criterion for differentiation. If the thrombocyte level is lower than 160 G/l the diagnosis of malaria and dengue is more likely. Most of the malaria patients have thrombocytes less than 120 G/l. H1N1 or dengue infections are more improbable if CRP is above 50 mg/l. Presented data may help for early differentiation between H1N1, dengue and malaria infections. However, the standard diagnostic procedures are still essential.

2 Zusammenfassung

2.1 Zielsetzung

In infektiologischen Notfallambulanzen und Schwerpunktkrankenhäusern werden immer wieder Reiserückkehrer mit akut fieberhaften Erkrankungen vorstellig. Dabei handelt es sich häufig um Plasmodien-, Dengue- oder Influenza-Infektionen. Aufgrund der relative geringen Sensitivität und Spezifität der Dengue – und Influenza-Schnelltestdiagnostik in der Frühphase der Erkrankungen, lassen sich beide Erkrankungen initial oft nicht diagnostizieren. Erst im weiteren Verlauf oder mit Hilfe von PCR- bzw. Schnelltest Untersuchungen kann dann die entsprechende Verdachtsdiagnose bestätigt oder ausgeschlossen werden.
Ziel dieser Untersuchung war es, im frühen Erkrankungsstadium laborchemische sowie klinische Unterschiede zwischen Dengue- und Influenza-Infektionen aufzuzeigen. Anhand dieser Unterschiede könnten frühzeitig Patienten für empirische Therapien (z.B. mit Neuraminidaseinhibitoren) selektioniert und auch präventive Maßnahmen (z.B. Heimquarantäne) rechtzeitig empfohlen werden.

2.2 Methoden

Es wurden retrospektiv die Daten von insgesamt 761 Patienten mit PCR bestätigten H1N1 Influenza Infektionen, mit Schnelltest bestätigten Dengue Infektionen und mikroskopisch bestätigten Plasmodien Infektionen im Zeitraum vom 1. Januar 2005 bis 1. Juni 2013 ausgewertet. Die PCR Untersuchungen wurden am Institut für Hygiene, Mikrobiologie und Umweltmedizin und am Institut für Krankenhaushygiene, LKH Universitäts-Klinikum Graz durchgeführt. Die Dengue-Schnelltest wurden ebenfalls am Institut für Hygiene, Mikrobiologie und Umweltmedizin, am Institut für Krankenhaushygiene, LKH Universitäts-Klinikum Graz oder am Departement für Virologie Wien, Medizinische Universität Wien durchgeführt. Die Malaria Blutausstriche wurden in den lokalen Labors ausgeführt.
Die Datenerhebung erfolgte durch die steirische elektronische Krankenhausdatenbank – MEDOCS. Teilnehmende Krankenhäuser waren das LKH Universitätsklinikum Graz und die Landeskrankenhäuser Graz West, Leoben, Bruck, Feldbach, Rottenmann, Bad Radkersburg, Hartberg, Hörgas-Enzenbach, Voitsberg und Judenburg/Knittelfeld. Schlussendlich wurden die Daten von 287/761 Patienten mit bestätigter H1N1-, Dengue- oder Malaria Infektion

analysiert. Die Daten von den restlichen 474 (62%) Patienten waren im MEDOCS nicht verfügbar.

Die Daten von H1N1-, Dengue- und Malaria Patienten wurden in Bezug auf klinische Präsentation, laborchemischen Parametern und vorbestehenden Grunderkrankungen analysiert.

2.3 Resultate

Das mittlere Alter in der H1N1 Gruppe betrug 16 Jahre im Vergleich zu 33 Jahren in der Dengue- und 39 Jahren in der Malaria Gruppe ($p<0,001$). 27% der H1N1 Patienten und 26% der Dengue Patienten wurden nur ambulant vorstellig im Vergleich zu 6% der Malaria Patienten, wohingegen 90% der Malaria Patienten stationär aufgenommen wurden, im Gegensatz zu 74% der Dengue- und 66% der H1N1 Patienten ($p=0,007$). Die mittlere Dauer zwischen dem Auftreten erster Symptome und ambulanter Vorstellung war 1 (IQR 0-2) Tag in der H1N1 Gruppe versus 4 (IQR 1-5) Tage in der Dengue- und ebenfalls 4 (IQR 2-4) Tage in der Malaria Gruppe ($p<0,001$). Das plötzliche Auftreten von Symptomen trat in 82% der H1N1-, in 95% der Dengue- und in 96% der Malaria Patienten auf ($p<0,015$). Fieber kam in 93% der H1N1-, sowie in 93% der Dengue Patienten und in 81% der Malaria Patienten ($p=0,038$).

H1N1 Patient hatten vermehrt folgende respiratorische Symptome: Husten (73% versus 15% bei Dengue und 10% bei Malaria; $p<0,0001$), Schnupfen (24% versus keine Dengue bzw. Malaria Patienten; $p<0,0001$), Giemen (14% versus keinen Dengue bzw. Malaria Patienten, $p<0,001$), Dyspnoe (22% versus 4% der Malaria- und keinen Dengue Patienten; $p<0,0001$), Brustschmerz (9% versus 4% der Malaria- und keinen Dengue Patienten), Rasselgeräusche (10% versus keinen Dengue- und Malaria Patienten), Halsschmerzen (12% versus 8% der Dengue- und 2% der Malaria Patienten) und 19% der H1N1 Patienten hatten Infiltrate im Thorax Röntgen im Vergleich zu 5% der Dengue- und keinen Malaria Patienten.

Dengue Patienten präsentierten sich häufiger mit Hautausschlag (36% im Vergleich zu 3% bei H1N1 und niemandem bei Malaria; $p<0,0001$), Myalgie (49% versus 24% bei H1N1 und 18% bei Malaria; $p=0,004$), Diarrhoe (44% versus 11% der H1N1- und 10% der Malaria Patienten; $p<0,0001$), Kopfschmerzen (67% versus 33% der H1N1- und 39% der Malaria Patienten; $p<0,0001$),

Bauchschmerzen (23% versus 6% der H1N1- und 6% der Malaria Patienten; p=0,008).

Keine Signifikanz bestand in Bezug auf Übelkeit/ Erbrechen (25% der H1N1-, 26% der Dengue- und 37% der Malaria Patienten) und Konjunktivitis (4% der H1N1-, 3% der Dengue- und 4% der Malaria Patienten).

Hinsichtlich vorbestehender Grunderkrankungen konnten wir einen signifikanten Unterschied bei Asthma bronchiale (10% der H1N1- versus 2% der Malaria- und keinen Dengue Patienten; p=0,026) und neurologischen Erkrankungen (10% der H1N1- versus keinen Dengue- bzw. Malaria Patienten; p=0,009) feststellen.

Bei den Laboruntersuchungen unterschieden sich folgende Parameter signifikant: Leukozyten Konzentration: 6,74 G/l (IQR 5-8,61) in der H1N1 Gruppe, 5,11 G/l (IQR 3,06-7,46) in der Dengue Gruppe und 5,86 G/l (IQR 4,52-6,94) in der Malaria Gruppe (*p=0,002*); Thrombozyten Konzentration: 92 G/l (IQR 65,00-115) in der Malaria Gruppe, 204 G/l (IQR 166-243) in der H1N1 Gruppe und 172 G/l (IQR 118-229) in der Dengue Gruppe *(p<0,0001)*; CRP Konzentration: 98,7 mg/l (IQR 55,95-152,5) in der Malaria Gruppe im Vergleich zu 17,4 mg/l (IQR 5,5-43,5) in der H1N1 und 13 mg/l (IQR 3 -36,2) in der Dengue Gruppe (*p<0,0001*); Gesamt-Bilirubin Konzentration: 1,24 mg/dl (IQR 0,93-2,34) in der Malaria Gruppe im Vergleich zu 0,4 mg/dl (IQR 0,29-0,67) in der H1N1 Gruppe und 0,43 mg/dl (IQR 0,36-0,62) in der Dengue Gruppe (p<0,0001); Gamma GT Konzentration: 72 U/l (IQR 34-140) in der Malaria Gruppe im Vergleich zu 25 U/l (IQR 15-49) in der H1N1 Gruppe und 46,5 U/l (IQR 27-84) in der Dengue Gruppe *(p<0,0001)*; AST Konzentration: 29 U/l (IQR 24-43) in der H1N1 Gruppe im Vergleich zu 48,5 U/l (IQR 30-87) in der Dengue Gruppe und 44 U/l (IQR 33-63) in der Malaria Gruppe (p<0,0001); ALT Konzentration: 20 U/l (IQR 15-34) in der H1N1 Gruppe im Kontrast zu 43,5 U/l (IQR 28-93) in der Dengue Gruppe und 44 U/l (IQR 25-70) in der Malaria Gruppe (*p<0,0001*); LDH Konzentration: 305,5 U/l (IQR 243-414) in der Malaria Gruppe im Vergleich zu 223 U/l (IQR 178-270) in der H1N1 Gruppe und 267 U/l (IQR 204-394) in der Dengue Gruppe (*p<0,0001*).

2.4 Zusammenfassung

Der plötzliche Beginn von Symptomen ist nicht spezifisch für Influenza-Erkrankungen und wird ebenso häufig bei Malaria und Dengue Patienten

beobachtet. Respiratorische Symptome wie Husten, Schnupfen, Giemen, Dyspnoe und Brustschmerzen treten jedoch deutlich häufiger bei H1N1 Patienten auf und werden sehr selten bei Patienten mit Dengue oder Malaria festgestellt. Diarrhoe, Bauchschmerzen und andere abdominelle Auffälligkeiten scheinen dagegen deutlich häufiger bei Dengue Erkrankungen zu sein und sprechen gegen eine Influenza Infektion.

Leukopenie kommt in allen drei Gruppen vor und ist daher kein brauchbares Kriterium zur Unterscheidung. Bei jeglicher Thrombopenie (Thrombozyten unter 160 G/l) ist eine Influenza Infektion unwahrscheinlich und eine Malaria bzw. Dengue Infektion sollte differentialdiagnostisch im Vordergrund stehen. Bei einer Malaria findet sich meist eine ausgeprägte Thrombopenie von unter 120 G/l. H1N1 bzw. Dengue Infektionen sind bei einem CRP über 50 mg/l eher unwahrscheinlich.

Unsere Daten könnten dazu beitragen, frühzeitig zwischen Influenza, Dengue und Malaria Infektionen zu unterscheiden. Die übliche Standarddiagnostik bleibt jedoch unverzichtbar.

3 Fever in Returning Travelers

Every year approximately 80 million people travel to developing countries, 15% to 64% return with self reported health problems and up to 8% are ill enough to require medical care while abroad or after returning home (1-5). Fever appears in 2-3% of returning travelers (3,4) and may present as a mild, self-limited illness (4,5).

The evaluation of these patients is very important and should consist of basic information, geographic distribution (where the person has traveled) and activities conducted while traveling (5-7). According to Freedman et al. specific travel destinations are associated with a higher probability of the diagnosis of certain diseases (4,5). Thus, travelers returning from sub-Saharan Africa and south-central/ Southeast Asia are more likely to present febrile illnesses (4-6).

3.1 Epidemiology and Etiology

Principal manifestations for 67% of ill returning travelers fell into 4 major syndrome categories (5) and occurred disproportionately in travelers returning from following regions:

- Systemic febrile illness without localizing findings (sub-Saharan Africa or Southeast Asia)
- Acute diarrhea (south central Asia)
- Dermatologic disorders (Caribbean or Central or South America)
- Chronic diarrhea

The specific etiologies for systemic febrile illness were as follows:

- Malaria was the most common diagnose among patients with fever (27-42%) (4,6,8,9)
- Dengue was the other most common etiology (6-8%) causing fever (4,6,8). The occurrence of dengue is estimated to be more frequently, due to dengue's mild and non-specific symptoms.

- 22% had an unspecified febrile illness
- Respiratory infections including influenza occurred in 14% of febrile patients
- Acute febrile diarrheal disease in 15% (6)

The majority of patients (almost 70%) who presented at GeoSentinel sites did travel to sub-Saharan Africa, Southeast Asia, the Caribbean and Central and South America (6). The substantial causes of fever were the following:

- Sub-Saharan Africa: 41% of ill returning travelers presented with fever and 49% with a systemic febrile illness with malaria (49%) as the main cause (dengue in 1%, respiratory illness in 10% and unspecific fever in 19%)
- Southeast Asia: Fever occurred in 33% of ill returning travelers and 34% presented with systemic febrile illness with dengue (18%) being the main etiology (malaria in 7%, no diagnosis in 22% and respiratory illness in 17%)
- Caribbean, Central and South America: 18% of ill returning travelers reported fever and 25% had a systemic febrile illness (dengue in 9%, malaria in 8%, respiratory illness in 13% and no diagnosis in 26%)

Table 1 Regional distribution of febrile ill returning travelers and major diagnosis groups (adapted from (6))

Region of travel	Fever	Systemic febrile illness	Malaria	Dengue	Undiagnosed febrile illness	Respiratory illness
Sub-Saharan Africa	41%	49%	42%	1%	19%	10%
Southeast Asia	33%	34%	34%	7%	18%	17%
Caribbean, Central and South America	18%	25%	8%	9%	26%	13%

3.2 Clinical History

A good clinical history is always very important, especially in ill returning travelers and should include the duration of travel, travel destinations (every region and country visited as well as transit stops and layovers), a day-by-day itinerary (exact dates of travel to every locale), exposures (detailed dietary history, fresh water, new sexual partners) (10), type of travel (accommodation, budget travel, activities, transportation), chemoprophylaxis (antimalarial drug), vaccinations as well as the time of onset of symptoms. The clinician should know about the most common infectious diseases in returning travelers and their likelihood in terms of knowledge of regional distributions, risk factors, exposures and the usual and typical incubation periods of these diseases (4).

3.3 Initial evaluation

3.3.1 Physical examination

Physical findings in ill returning travelers are often non-specific and present with undifferentiated fever without localizing findings. The clinician should pay attention to skin lesions (e.g. rash), lymphadenopathy, hepatomegaly, and splenomegaly, neurological findings and respiratory signs in the physical examination (4,11).

3.3.2 Laboratory Tests

The minimal initial laboratory work-up should include: complete blood count (with differential and liver enzymes), thick and thin blood smears for malaria and blood cultures (11).

3.4 Differential diagnosis - Considerations for the common travel-related febrile illnesses

The differential diagnosis in ill returning travelers should comprise common febrile diseases and those with a worldwide distribution (such as influenza (12)) as well as typical tropical infections such as malaria and dengue fever. As mentioned above ("Epidemiology and Etiology", 3.1) malaria, dengue, respiratory infections including influenza and unspecified fever were the most common diseases in returning travelers (acute diarrheal diseases excluded) (6).

3.4.1 Malaria

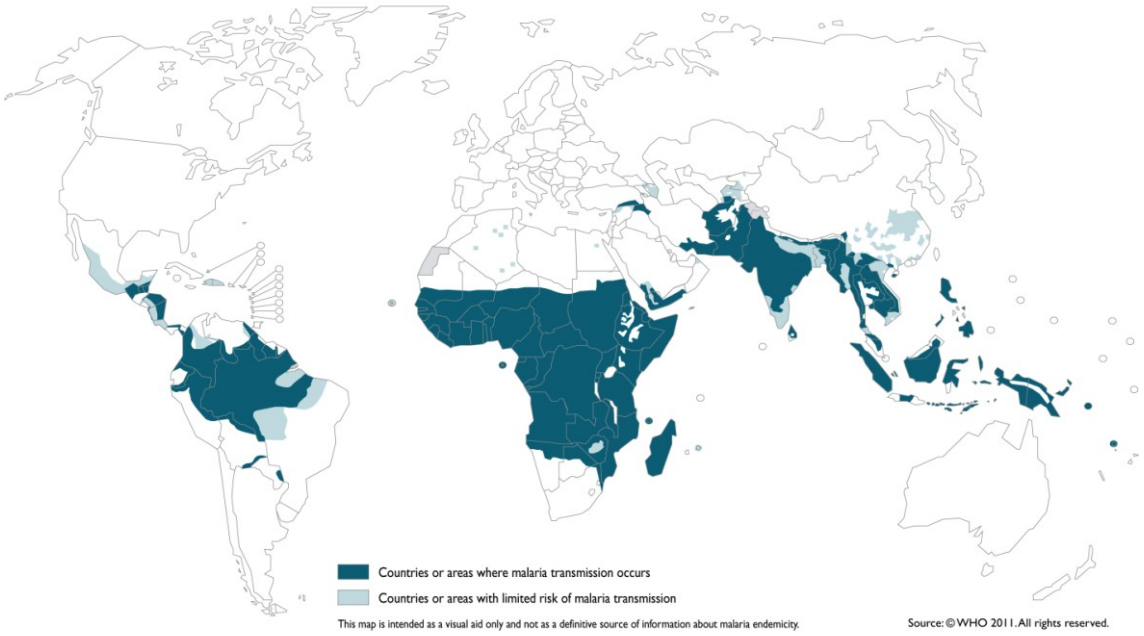
Malaria is an acute febrile illness and is the most common tropical infection in travelers and the most important of all tropical parasitic diseases (6,13).
In 2010, the WHO estimated 219 million cases of malaria with 660,000 deaths worldwide. The malaria mortality rates have decreased by more than 25% globally since 2000 and 33% in WHO African region. Every minute a child is dying in Africa due to malaria (14).

Malaria is caused by protozoan parasites of the genus plasmodium with six human pathogenic species (15):

- P. falciparum (most deadly)
- P. vivax
- P. ovale (two species)
- P. malariae
- P. knowlesi (a primate parasite in Southeast Asia)

Plasmodium vivax and Plasmodium falciparum are the most common (14).

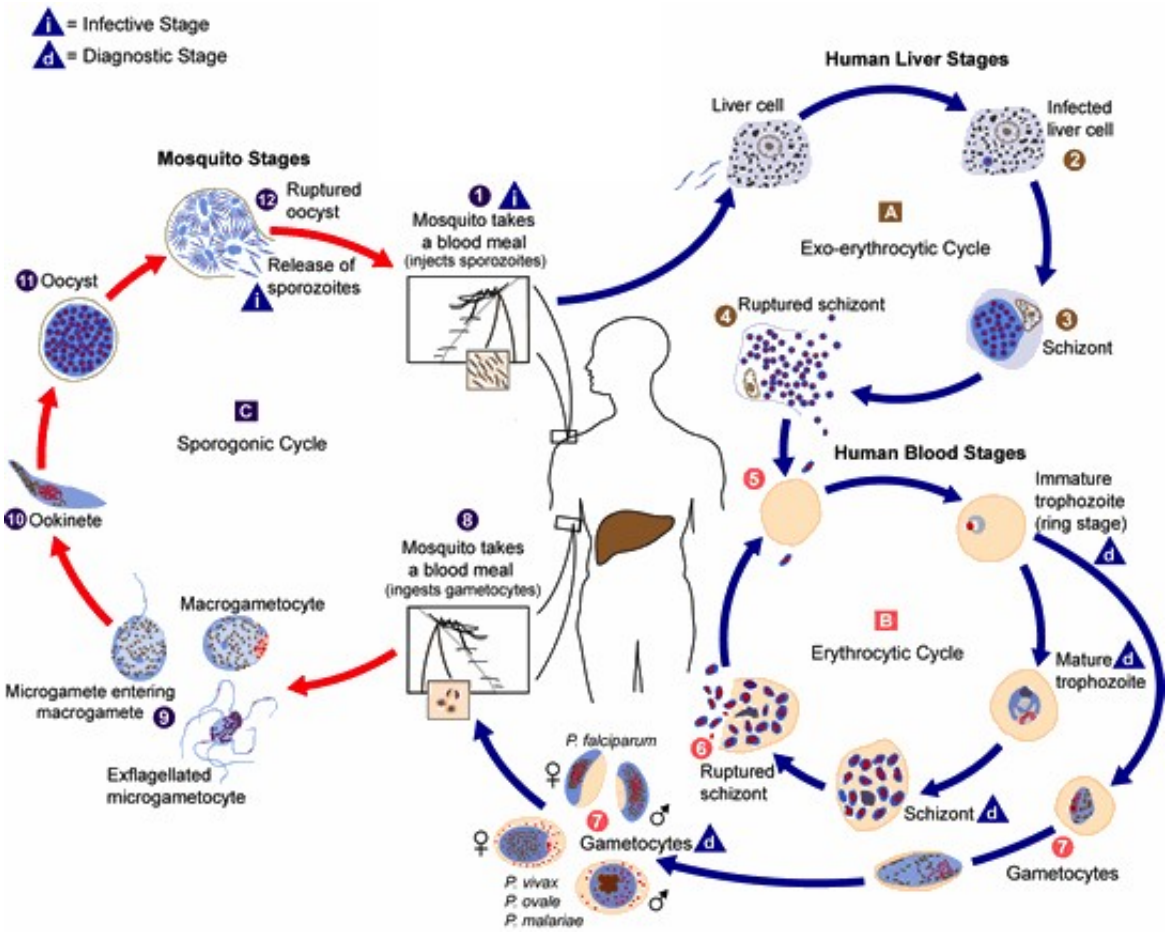
Figure 1 Malaria – countries/ areas at risk of transmission, 2008 (adapted from (16))



3.4.1.1 Transmission

Malaria is transmitted by the bite of an infected anopheline mosquito. Sporozoites are injected into humans during a blood meal of an anopheles mosquito and invade hepatocytes. After schizogony (asexual multiplication of parasites) in liver cells or erythrocytes, merozoites are released into the blood stream and penetrate red blood corpuscles (RBC) where further asexual multiplication happens before gametocytes form. If a female mosquito ingests male and female gametocytes, they develop further in her stomach, resulting in ookinetes leading to a release of daughter sporozoites. Hypnozoites (P. vivax and P. ovale only) may remain in the liver and cause later relapse of parasitemia and symptoms (13,15).

Figure 2 Life cycle of malaria parasite (adapted from (17))



3.4.1.2 Symptoms

Symptoms appear seven days or more (10-15 days) after an infective mosquito bite. They can be mild and non-specific and comprise fatigue, muscle and joint aches, fever, headache, nausea/vomiting, anorexia, chills and worsening malaise. Sever malaria (especially caused by P. falciparum) can present with the following symptoms: cerebral malaria, severe anemia, respiratory distress, metabolic acidosis and abnormal bleeding, jaundice and multi-organ involvement. Asymptomatic infection may occur in persons with partial immunity (in endemic areas) (14,15,18).

3.4.1.3 Diagnosis and Treatment

Prompt and accurate diagnosis and early treatment of malaria reduce disease and prevent deaths (14,18).

Thick and thin blood smears repeatedly over a period of 72 hours are necessary to confirm the diagnosis of malaria. Malaria rapid diagnostic tests (RDT) detect parasite-specific antigens or enzymes and most of them are sensitive and specific for P. falciparum, but are more useful where skilled microscopy is unavailable (13,15).

Malaria caused by P. vivax, P. ovale and P. knowlesi is treated with chloroquine. P. falciparum malaria is best treated with an artemisinin-based combination therapy (ACT) (14). The WHO recommends the combination of two or more different antimalarial drugs due to emerging resistance of P. falciparum (15).

3.4.1.4 Prevention

Vector control is the main way and the only intervention that can reduce malaria transmission. Personal protection with repellents such as diethyltoluamide (DEET) is the first line of defense for malaria prevention.

Two forms are effective in terms of modern malaria control (14,15):

- Insecticide-treated mosquito nets (ITNs)
- Indoor spraying with residual insecticides (IRS)

Prevention in Travelers – Travelers are advised to (15):

- be aware of the risk
- prevent exposure (such as mentioned above) to anopheles mosquitoes
- chemoprophylaxis (if appropriate)
- seek medical care immediately if fever occurs

3.4.2 Dengue

3.4.2.1 Flaviviruses

There are more than 60 species in the Flavivirus genus of which about 30 are known to be human pathogenic. The most important Flaviviruses that are pathogenic for humans are Yellow Fever, Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), Japanese Encephalitis, West Nile Encephalitis and most common in Europe, the Tick-Borne Encephalitis. Especially in returned travelers the consideration of Flavivirus infection is important in the differential diagnosis of flu-like illnesses, hemorrhagic fever, central nervous system (CNS) infection and acute febrile illnesses with arthropathy or rash (19).

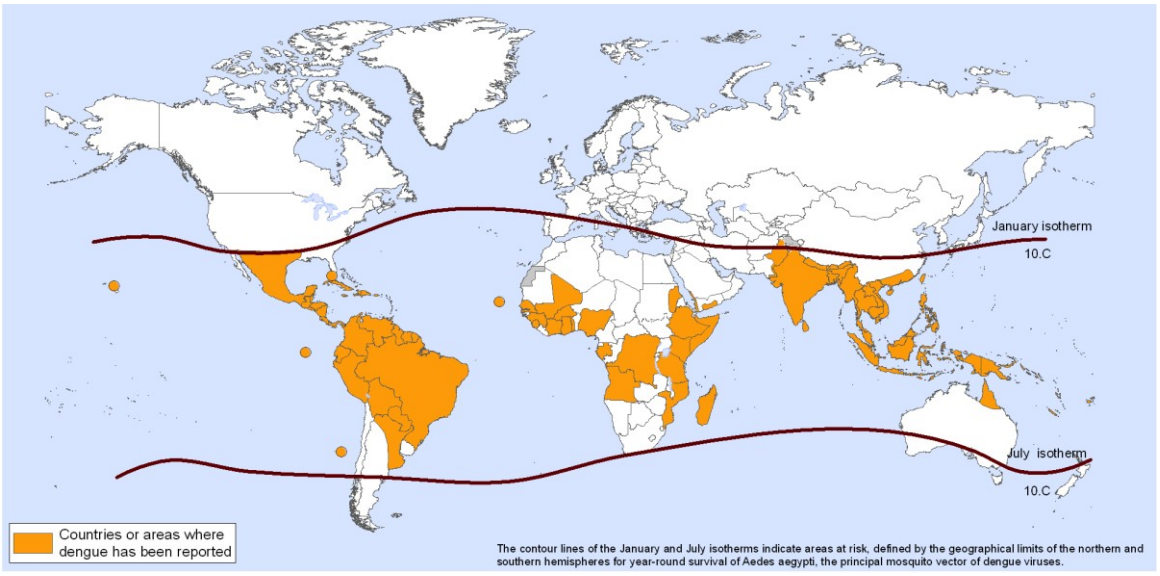
3.4.2.2 History of Dengue

There are no historical records of past dengue fever epidemics, due to its nonspecific symptoms. B. Rush was the first to describe dengue fever as break-bone fever in 1780 (19,20). Desultory outbreaks in the tropics and subtropics were reported. The transmission via mosquitos was demonstrated in 1903 and the detection of different serotypes of the virus in 1944 (19). In the 1920s epidemics in Greece occurred and after World War II the beginning of an intensified transmission of multiple viral serotypes in Southeast Asia was recognized, which resulted in outbreaks of dengue hemorrhagic fever (DHF) (19,21).

3.4.2.3 Epidemiology

The incidence of the dengue virus is worldwide, but mainly appears in the tropics, in an area between latitudes 35°N and 35°S (19,22). Dengue virus infection is endemic and epidemic in over 100 countries in Africa, the Americas, the Caribbean, the Eastern Mediterranean, Southeast Asia and the Western Pacific regions (23). It is estimated that Dengue infection affects approximately 50 million people every year and about 2,5 billion people live in endemic countries, a third of the world population (22,24). It is assumed to be the most common viral disease in returning travelers from the tropics (25).

Figure 3 Countries /areas at risk of dengue transmission, 2006 (adapted from (16))



3.4.2.3.1 Dengue Virus (DENV)

The Dengue Virus belongs to the genus Arbovirus ("arthropod-borne") and is a small positive single stranded RNA virus (22). There are four closely related serotypes known as DENV 1 to 4 (22,25). DENV 1 Infection generates lifelong immunity against reinfection with DENV 1, but there is no long-term cross-protection against the other 3 DENV types (23). Among the 4 serotypes, the "Asian" genotypes of DENV 2 and DENV 3 are associated with severe illness accompanying secondary dengue infections (22).

3.4.2.3.2 Transmission Cycle

The vector of the DENV is the mosquito genus Aedes. The principle vector is Aedes aegypti, which is found worldwide in the tropics and subtropics, but other species like Aedes albopictus and Aedes polynesiensis can transmit the virus as well (19,24).

Transmission principally occurs after a female Aedes aegypti has bitten on a viremic human (23). The female mosquito ingests the DENV this way and the virus infects the mosquito mid-gut, passing through viral replication over a period of 8-12 days (19,22). After this extrinsic incubation period, the mosquito can transmit the virus to other humans during subsequent feeding attempts several times a

day. The mosquito remains infected for the rest of its lifetime (1-4 weeks) (19,22). Aedes aegypti oviposit in uncovered water storage containers, close to human dwellings. They bite in the morning and in the afternoon for an interval of 1-2 hours (19).

3.4.2.4 Pathogenesis

The pathogenesis of dengue hemorrhagic fever and dengue shock syndrome is poorly understood (26,27). Currently there are two theories attempting to explain the mechanisms of DHF and DSS.

- Antibody-dependent enhancement (ADE) theory:

This hypothesis proposes that residual antibodies bind to the new dengue virus, but are not able to neutralize and inactivate this virus. Macrophage cells uptake the virus-antibody complex through Fc receptors. In these cells the virus amplifies viral replication, driving an immunopathogenic cascade through cytokine release, resulting in capillary leakage and coagulopathy (26,27).

- Viral strain differences

This theory suggests that increased virulence of a virus population with a special affinity to immune cells via release of cytokine results in hemorrhagic manifestations (27,28).

3.4.2.5 Clinical Features

Most dengue virus infections are asymptomatic, but cause a wide variety of illnesses ranging from inapparent infection, to mild febrile illness, and to sever and fatal diseases. In the past, symptomatic disease was separated into dengue fever and dengue hemorrhagic fever, but with the new World Health Organization (WHO) guidelines for dengue (2009), it is divided into dengue and severe dengue (26). Symptomatic dengue is primarily a disease of older children and adults. After an incubation period of 4-7 days, the illness begins abruptly and typically follows three phases – febrile, critical and recovery (22,26).

3.4.2.5.1 Febrile phase

There is a sudden onset of high-grade fever and the acute febrile phase usually lasts 2-7 days accompanied by facial flushing, skin erythema, generalized body ache, myalgia, arthralgia and headache (22,26,29). Sore throat, injected pharynx and conjunctiva infection as well as anorexia, nausea and vomiting can occur (22). The clinical differentiation of dengue from non-dengue febrile diseases is difficult in the early phase; a positive tourniquet test increases the probability of differentiation (22,30). Thrombocytopenia, leukopenia and a slight increase of hepatic transaminases can be laboratory findings in the first week (26).

3.4.2.5.2 Critical phase

The beginning of the critical phase is marked by an increase in capillary permeability accompanied with increasing hematocrit levels, generally on day 3-7 at time of defervescence. Increased capillary permeability manifests with increasing hemoconcentration, hypoproteinemia, pleural effusions and ascites and if progress may develop the potentially life-threatening dengue shock syndrome (DSS). DSS is defined as having a pulse pressure of less than 20mmHg with a rapid weak pulse and impaired peripheral perfusion. If warning signs (severe vomiting, intense abdominal pain and increasing tender hepatomegaly) occur, that means the patient will deteriorate to severe dengue. In this phase, fluid resuscitation is life saving and leads to a full recovery (22,26).

3.4.2.5.3 Recovery phase

The patient improves after surviving the 24-48 hour critical phase, when permeability reverts to normal and fluid is reabsorbed quite rapidly. Some patients may have a rash on day 6 to 7, with intense pruritus (22,26).

Table 2 Febrile, critical and recovery phase in dengue (adapted from (22))

1	**Febrile phase**	Dehydration, high fever
2	**Critical phase**	Shock from plasma leakage, severe hemorrhage, organ impairment
3	**Recovery phase**	Hypervolemia (only if excessive rehydration occurred)

3.4.2.5.4 Severe dengue

One or more of the following symptoms define severe dengue (22,26):

- Plasma leakage resulting in shock and/or
- fluid accumulation with or without respiratory distress and/or
- severe bleeding and/or
- severe organ impairment (e.g. liver failure, myocarditis, etc.)

3.4.2.6 Laboratory Diagnosis

In the early febrile phase (up to about day 5 of illness) the detection of viral RNA by reverse transcription-polymerase chain reaction (RT-PCR) or NS1 (ELISA test for detection of circulating dengue nonstructural protein 1) are promising tools for early diagnosis (26,31).
After the early phase, ELISA tests with a rising titer of dengue-specific IgM or IgG indicates acute infection. Unfortunately, IgM is detectable in large amounts only after 4–5 days of infection (24). Serology diagnosis is complicated due to the existence of flavivirus cross-reactivity (26).

3.4.2.7 Therapy/Management and Vaccination

There is no specific therapy available for DENV infections. Good supportive care, especially careful fluid management, is crucial for dengue therapy. Oral fluid intake is essential to avoid dehydration.
Fever can be managed with tepid sponging and paracetamol. Aspirin and non-steroidal anti-inflammatory drugs are contraindicated due to risk of bleeding complications (26).

The feasibility of vaccine development is based on the fact, that infection with DENV provides long-term immunity against the particular serotype. Currently no licensed vaccine is available. A recombinant, live, tetravalent formulation of attenuated yellow fever 17D vaccine strain reduced the risk of dengue infection by only 30% among 4000 children in Thailand (32).

3.4.2.8 Prevention

The prevention of DENV infection still relies on biological and chemical mosquito control and elimination of potential vector breeding sites. Most effective personal protection is achieved in using DEET or picaridin as mosquito repellents and protective clothing (26).

3.4.3 Influenza (Respiratory Diseases)

There are three types of seasonal influenza – A, B, and C. Only types A and B are associated with the epidemics of influenza “seasons”. Type A is further divided into subtypes; currently H1N1 and H3N2 are circulating among humans. The viral envelope contains two glycoproteins, the haemagglutinin (H) and neuraminidase (N), which are critical in host immunity (33-35).

3.4.3.1 Epidemiology

Influenza viruses undergo mutational change and eventually results in the emergence of antigenic variants. Every few years, a variant causes a global epidemic (34).

The geographical distribution of influenza viruses is worldwide and seasonal epidemics occur during autumn and winter in temperate regions, causing three to five million cases of severe illness and about 250,000 to 500,000 deaths. In tropical areas influenza viruses circulate throughout the year with no clear seasonal pattern (33,35).

The 2009 pandemic arose in Mexico, caused by a novel H1N1 virus of swine-origin and rapidly spread around the world. The pandemic was associated with explosive outbreaks in children and young adults while there was less infection in older people. People born prior to 1950s had substantial cross-protection against the novel virus (34).

3.4.3.2 Transmission

Respiratory transmission occurs through direct contact, contaminated fomites and large airborne droplets disseminated by unprotected coughs and sneezes (33,34).

3.4.3.3 Clinical features

The severity of influenza diseases ranges from asymptomatic infection to fatal diseases. Typical symptoms comprise fever with sudden onset, chills, sore throat headache, nonproductive cough, coryza, myalgia and sometimes prostration. Fever usually lasts one to five days, with hyperemic pharynx and crackles or wheezing is heard in approximately 10% of patients.
Common complications of influenza include otitis media, exacerbation of underlying chronic conditions such as asthma, primary influenza viral pneumonitis and bacterial pneumonia (33,34).

3.4.3.4 Treatment and prevention/ vaccines

There are two classes of antiviral drugs for influenza:

- Adamantanes (amantadine and remantadine) – ion channel blockers interfering with viral uncoating
- Neuraminidase inhibitors (oselatmivir and zanamivir) – blocking virus release from infected cells.

Adamantanes are only active against influenza A, whereas neuraminidase inhibitors are also active against influenza B (34,35).

The most effective way to prevent influenza diseases is vaccination, preventing 70% to 90% of influenza-specific illness.
Influenza vaccine contains antigens from the two subtypes of human influenza A (H1N1 and H3N2) and B viruses. Influenza vaccine virus strains are modified every year to make sure the vaccine is matched as closely as possible to the currently circulating viruses, therefore annual reimmunization is required. This updating of the vaccine is achieved through the WHO Global Influenza Surveillance Network (GISN), which monitors the influenza viruses circulating in humans (33-35).

4 Material und Methods

4.1 Objectives

- To find clinical differences in patients with blood smear confirmed plasmodium infection, patients with positive dengue rapid diagnostic tests (IgM and/or IgG) and patients with PCR confirmed H1N1 influenza infection.
- To find laboratory differences in patients with blood smear confirmed plasmodium infection, patients with positive dengue rapid diagnostic tests (IgM and/or IgG) and patients with PCR confirmed H1N1 influenza infection.
- To describe the clinical management and treatment in confirmed malaria and dengue fever cases.

4.2 Study Design

This study was performed as a retrospective survey of patients with rapid diagnostic test confirmed dengue infection; PCR confirmed H1N1 influenza infection and blood smear confirmed plasmodium infection. The patients in the H1N1 influenza group with confirmed PCR were tested between the 1st of October 2009 and the 21st of January 2010; the patients with confirmed malaria and dengue infection were tested between the 1st of January 2005 and the 1st of June 2013. It was a multicenter study and participating hospitals were the Medical University Hospital of Graz and the state hospitals of Graz West, Leoben, Bruck, Feldbach, Rottenmann, Bad Radkersburg, Hartberg, Hörgas-Enzenbach, Voitsberg and Judenburg/Knittelfeld. Data sets were available from 199/624 (32%) of H1N1 infected patients, from 49/58 (84%) of malaria infected patients and from 39/79 (49%) of dengue infected patients. In certain cases the number of patients may vary due to unavailability of some data. The reasons for exclusion are listed in “4.3.2 Exclusion Criteria”.

H1N1 influenza PCR was performed at the Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz and at the Institute of Hospital Hygiene, University Hospital of Graz.
Dengue rapid diagnostic tests were performed at the Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, using the Panbio Dengue Duo Cassette test; at the Institute of Hospital Hygiene, University Hospital of Graz and at the Department of Virology, Medical University of Vienna. The Panbio Dengue Duo Cassette is an immunochromatographic test (=rapid diagnostic test) for the qualitative presumptive detection of IgM and IgG antibodies to dengue virus (serotypes 1-4) in human serum, plasma and whole blood. The assayed overall sensitivity and specificity for IgM detection is 65,3% and 97,6% (36).
Malaria blood smear was performed at the local laboratories.

The study was conducted at the Section of Infectious Diseases and Tropical Medicine, Division of Pulmonology, Medical University of Graz.
Patient's data was evaluated anonymously and via the Case Report Form by reviewing the medical reports in the MEDOCS database, the electronic Styrian Hospital Network. The local ethics committee granted the research ethic board approval. A priori patient consent was not required due to the non-interventional character of the study.

4.3 Patient Definition

4.3.1 Inclusion Criteria

- All patients who had rapid diagnostic test confirmed dengue infection, PCR confirmed H1N1 infection or blood smear confirmed plasmodium infection in Styrian Hospitals were included in this study.

4.3.2 Exclusion Criteria

- 407/624 (65%) of H1N1 patients, 4/58 (7%) of malaria patients and 35/79 (44%) of dengue patients were excluded from the study, because either there was no data or there was insufficient data available in the MEDOCS database. An

Example could be, that we received results of RDT confirmed dengue patients from the Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, but the data was not obtainable in the MEDOCS system.

- 8/624 (1,3%) of H1N1 patients and 1/58 (2%) of malaria patients were excluded, due to their severe underlying diseases (e.g. malignant diseases).
- 10/624 (1,65) of H1N1 patients, 4/58 (7%) of malaria patients and 5/79 (6%) of dengue patients were excluded when symptoms lasted for more than 7 days before admission to the hospital.

4.4 Group Stratification

- The stratification was performed as the following:
  - Dengue illness was defined as acute febrile disease, which was confirmed with positive dengue rapid diagnostic tests.
  - Malaria was defined as acute febrile disease with confirmed positive blood smear.
  - H1N1 influenza illness was defined as acute respiratory and/or febrile disease, which was confirmed by positive H1N1 influenza PCR.

4.5 The Case Report Form

The Case Report Form comprised:

- Age at diagnosis
- Gender
- Date of proofed disease
- Date of onset of symptoms
- Date of first clinical presentation
- Demographic Data
- Care unit
- Symptoms
- Clinical findings
- Parasite concentration

- Dengue IgM and IgG
- Antimalarial and antibiotic treatment
- Laboratory findings
- Radiological data
- Underlying medical condition
- Vaccination status

Furthermore, we calculated the time between the onset of symptoms and the presentation at the hospital, the duration of hospitalization, time between the onset of symptoms and PCR/ dengue RDT/ blood smear results. We also calculated the period of first presentation to PCR/ dengue RDT/ blood smear results and the time between the onset of symptoms and the start of antibiotic and/or antimalarial treatment.

4.6 Analysis

Statistical analysis was done via “IBM SPSS 15.0/ 21.0”. Data from H1N1 influenza, dengue and malaria was compared using the chi-square or the Mann Whitney U test. Continuous data is shown as medians [interquartile range 25-75 (IQR 25-75)]. Statistically significant data was considered a p-value less than 0,05.

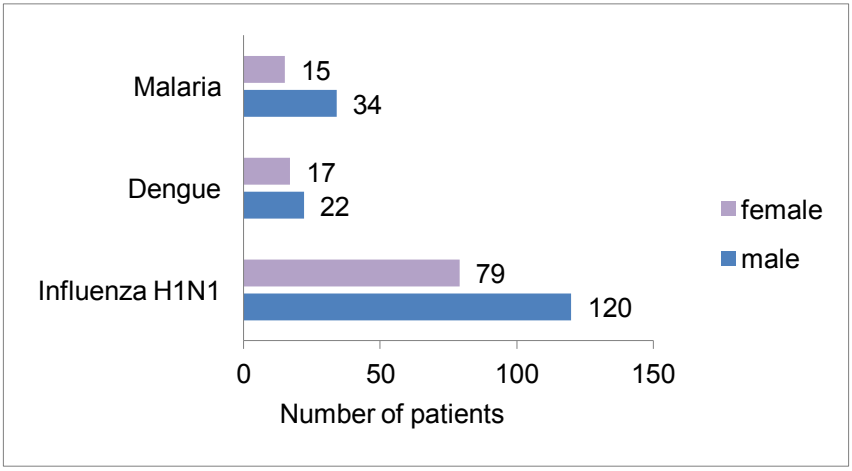
5 Results

Data from patients who presented to clinics in Styria between the 1st of January 2005 and the 1st of June 2013 have been reviewed via the MEDOCS system. The first positive result in the study was a patient with confirmed malaria who was observed on the 12th of January 2005 and the last patient with RDT confirmed dengue disease was observed on the 29th of May 2013.

5.1 Demographic Data

In general, H1N1 influenza, dengue and malaria were diagnosed more often in men than in women. Among the 287 total cases including H1N1, malaria and dengue, 176 (61%) patients were male and 112 (39%) patients were female. Comparing the three groups, gender distribution was observed to be almost equal in each group (see Figure 4).

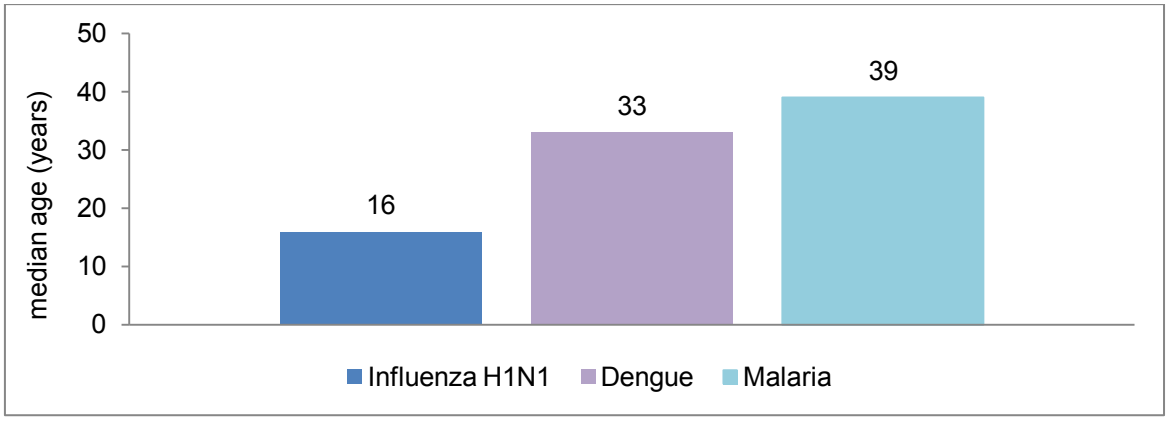
Figure 4 Gender distribution in H1N1 influenza, dengue and malaria.



The age at diagnosis in the H1N1 influenza group ranged from 0 to 79 years, in the dengue group the age ranged from 0 to 76 years and in the malaria group the age ranged from 0 to 61 years. Patients who suffered from H1N1 influenza (median age 16 years) were significantly younger than patients who suffered from

malaria (median age of 39 years) or dengue (median age 33 years) (*p<0,001*). (see Figure 5).

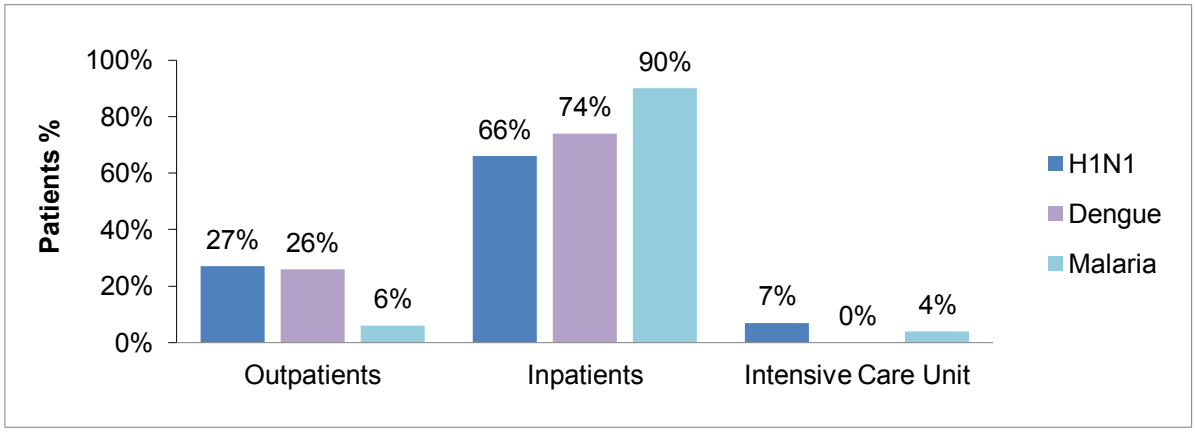
Figure 5 Median age in H1N1, dengue and malaria patients



5.2 Hospitalization

Among all patients with confirmed malaria only 3/49 (6%) presented to outpatient clinics, compared to 10/39 (26%) confirmed dengue patients and 53/199 (27%) confirmed H1N1 influenza patients. 44/49 (90%) patients with malaria were more frequently admitted to normal wards than 29/39 (74%) dengue patients or 131/199 (66%) H1N1 patients. 15/199 (7%) H1N1 patients on the other hand required intensive care as compared to 2/49 (4%) malaria patients and none of the dengue patients. The overall significant difference between the outpatient clinic, inpatient clinic and intensive care unit between the three groups was observed as *p=0,007*. (see Figure 6).

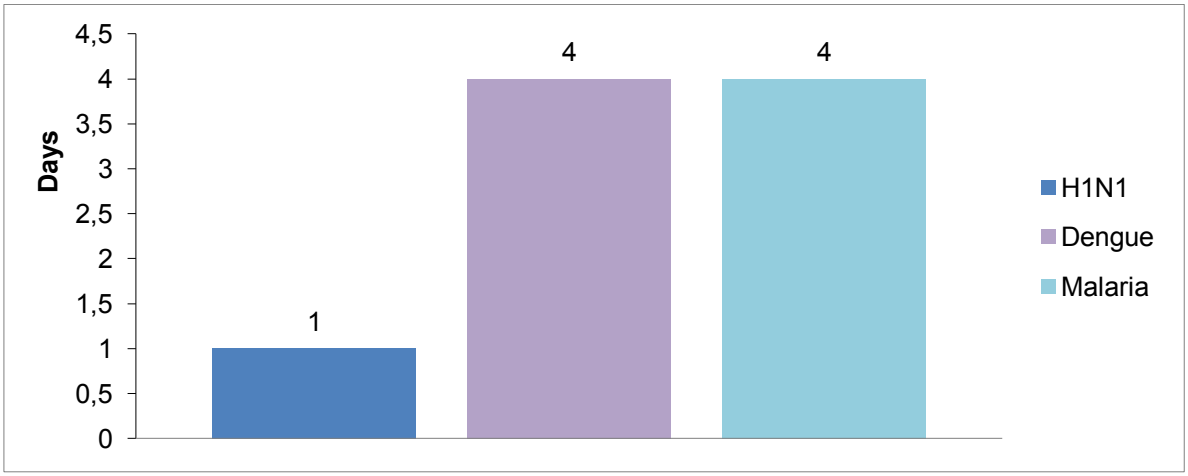
Figure 6 Percentage of patients who presented at outpatient clinic only or were hospitalized



5.3 Days between onset of disease and presentation at ER

The time between the sudden onset of disease and first presentation at emergency room/ outpatient clinic was observed with a statistically significant difference between patients with H1N1 and dengue or malaria *(p<0,001)*. The median time for patients with H1N1 was 1 day (IQR 0-2) compared to dengue with a median time of 4 days (IQR 1-5) and malaria with a median time of 4 days (IQR 2-4). (see Figure 7)

Figure 7 Days from onset of symptoms to first clinical presentation (median)



5.4 Anamnestic findings

5.4.1 Sudden Onset

A sudden onset of symptoms was found in 164/199 (82%) of H1N1 patients, in 37/39 (95%) of dengue patients and in 47/49 (96%) of malaria patients (*p=0,015*).

Table 3 Disease onset

	H1N1 Influenza	Dengue	Malaria	p-value
Sudden Onset of Symptoms	164/ 199 (82%)	37/39 (95%)	47/49 (96%)	0,015

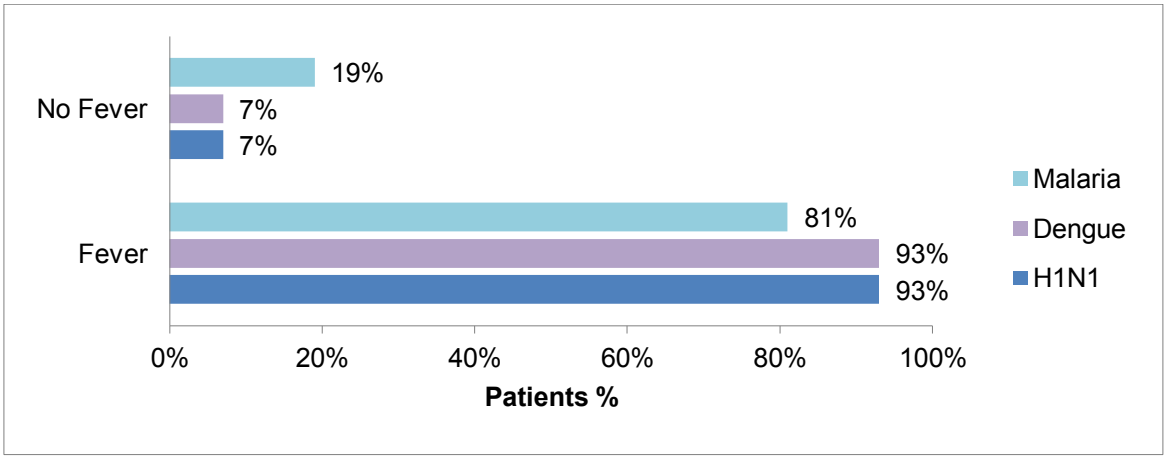
5.5 Clinical Presentation

5.5.1 Reported or measured Fever

Fever was defined as a temperature over 37,2° Celsius axillar, over 37,8° Celsius sublingual and rectal over 38,3° Celsius. Fever was the most frequent symptom in all three groups.

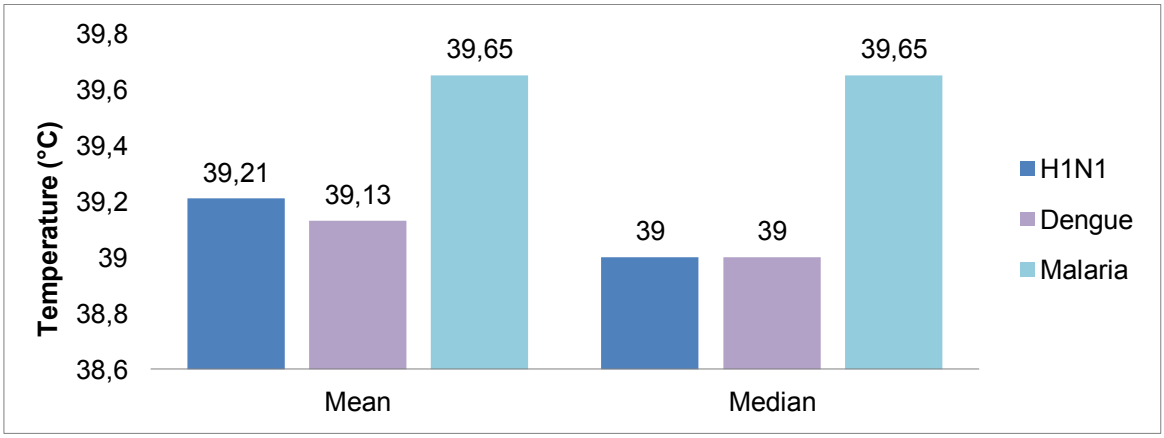
185/199 (93%) of H1N1 patients and 36/39 (93%) of dengue patients mentioned to have had fever at home or/and at the time of hospital admission, compared to 39/48 (81%) of malaria patients *(p=0,038)*. (see Figure 8)

Figure 8 Percentage of H1N1, dengue or malaria patients who presented with/no fever



The mean maximal temperature measured at home was 39,65°C (median 39,65°C) in the malaria group, 39,13°C (median 39,0°C) in the dengue group and 39,21°C (median 39,0°C) in the H1N1 group (p=0,026). (see Figure 10).

Figure 9 Mean and median body temperature at home



5.5.2 Rash

A statistically significant difference between the three groups was observed in respect to the rash. 14/39 (36%) suffering from dengue disease presented with rash compared to 0/49 (0%) of patients from the malaria group and 6/199 (3%) from the H1N1 group (*p<0,0001*). (see Table 4).

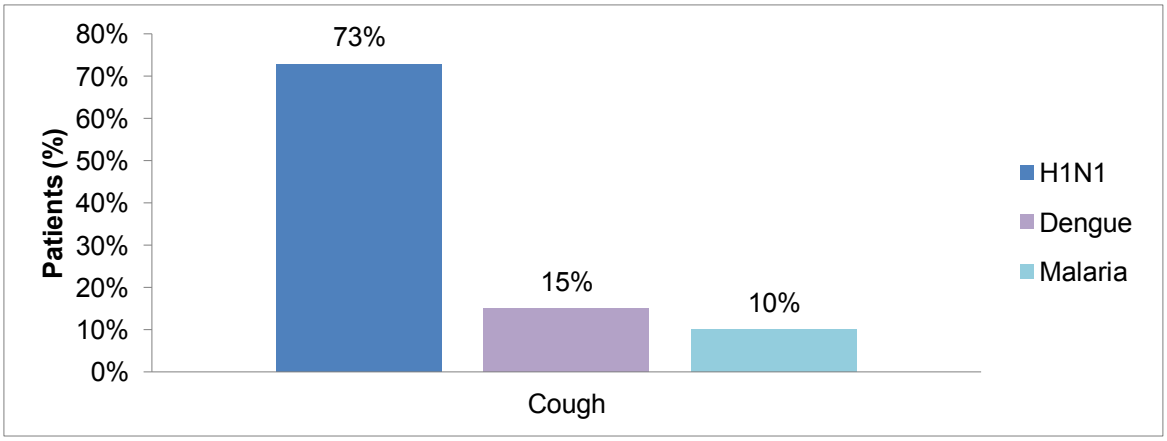
Table 4 Rash in dengue and malaria patients

	H1N1 (n=199)	Dengue (n=39)	Malaria (n=49)	p-value
Rash	6/199 (3%)	14/39 (36%)	0/49 (0%)	<0,0001

5.5.3 Cough

In respect of the symptom cough, a statistically significant difference was observed. In the H1N1 group 146/199 (73%) of patients mentioned to suffer from coughing compared to 6/39 (15%) of patients in the dengue group and 5/49 (10%) of patients in the malaria group (*p<0,0001*).

Figure 10 Percentage of H1N1, dengue and malaria patients who presented with cough

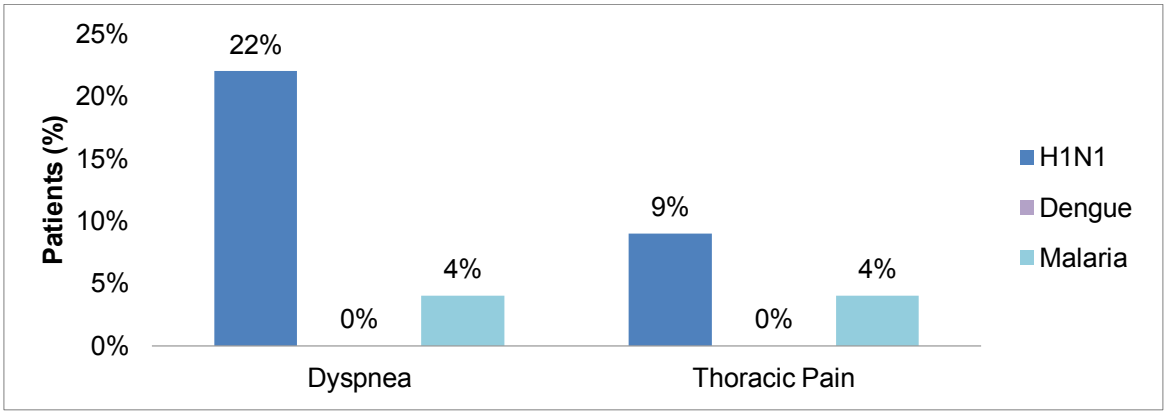


5.5.4 Dyspnea and Thoracic Pain

Concerning the dyspnea, a significant difference between the H1N1 group compared to the dengue and malaria group was found. In the H1N1 group 41/199 (22%) of patients suffered from dyspnea in comparison to 2/49 (4%) of patients in the malaria group and 0/39 (0%) of patients in the dengue group (*p<0,0001*).

No statistically significant difference was found in respect to thoracic pain. 17/199 (9%) of H1N1 patients, 2/49 (4%) of malaria patients and none of the dengue patients showed thoracic pain. (see Figure 11).

Figure 11 Dyspnea and thoracic pain in patients with H1N1, dengue or malaria.



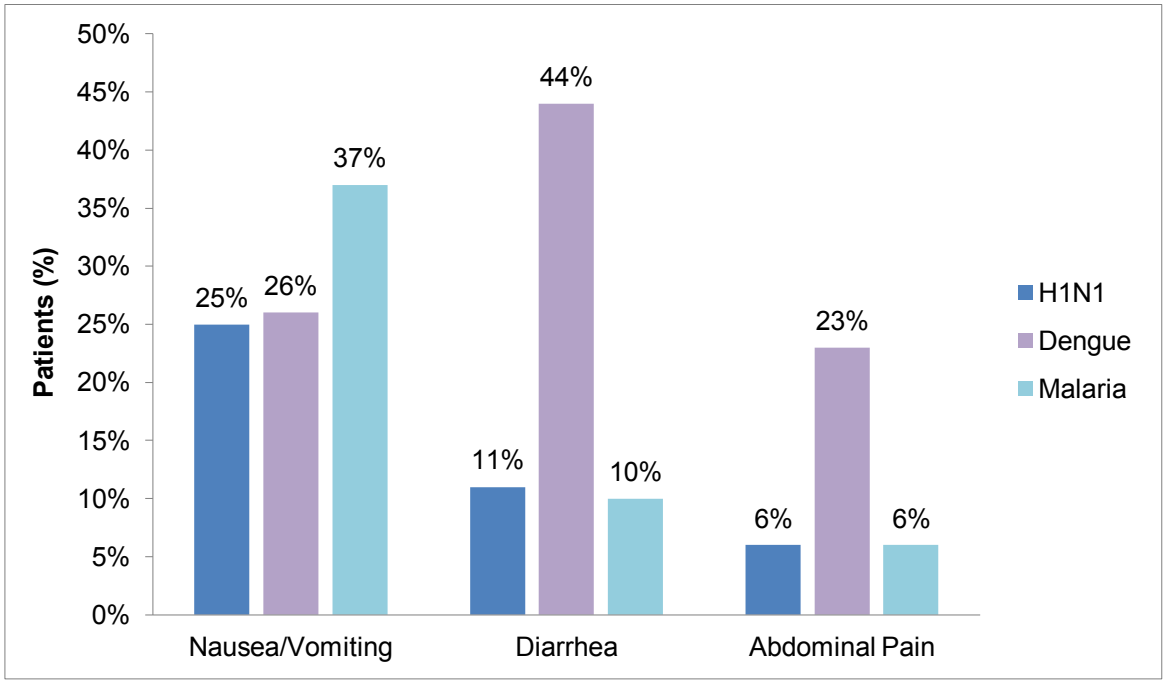
5.5.5 Gastrointestinal symptoms (Diarrhea, Nausea/Vomiting Abdominal Pain)

Nausea and/or Vomiting were the most common gastrointestinal symptoms observed, but with no statistically significant differences. 18/49 (37%) of malaria patients, 10/37 (26%) of dengue patients and 49/199 (25%) of H1N1 patients presented with nausea and/or vomiting at home and/or at hospital admission.

In respect to diarrhea we could find a significant difference (*p<0,0001*) in the dengue group [17/39 (44%) of patients] compared to the malaria [5/49 (10%) of patients] and the H1N1 group [21/199 (11%) of patients)].

As well as in respect to abdominal pain, we could find a difference in the three groups. Abdominal pain was more common in the dengue group, than in the H1N1 and malaria groups [9/39 (23%) versus 12/199 (6%) and 3/49 (6%); *p=0,008*]. (see Figure 12)

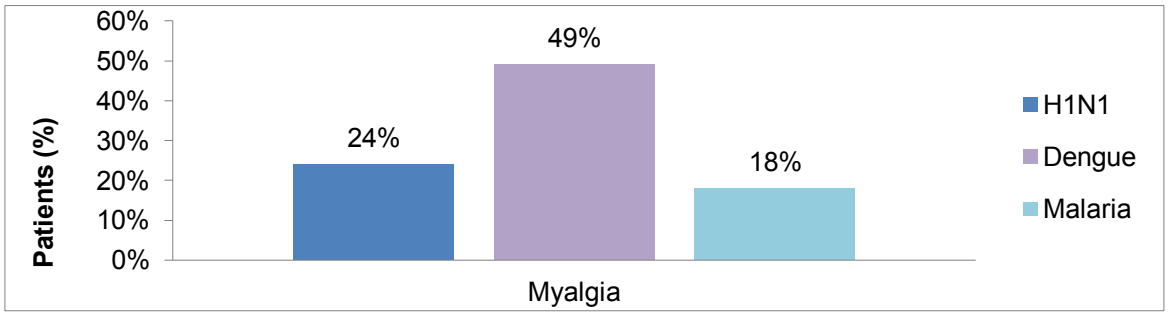
Figure 12 Patients with Nausea/Vomiting, Diarrhea and Abdominal Pain



5.5.6 Myalgia

Myalgia was most common in patients with dengue fever and significantly different compared to patients with H1N1 or malaria [19/39 (49%) versus 48/199 (24%) versus 9/49 (18%); *p=0,004*]. (see Figure 13)

Figure 13 H1N1, dengue and malaria patients with myalgia



5.5.7 Other symptoms (Rhinitis, Fatigue, Headache, Sore Throat)

Rhinitis was observed to occur only in the H1N1 group compared to dengue and malaria. 48/199 (24%) of H1N1 patients presented with rhinitis and neither the malaria nor the dengue group patients presented with rhinitis (*p<0,0001*).

In the H1N1 group fatigue seemed to occur more often than in the dengue or the malaria groups [120/198 (61%) of H1N1 patients versus 15/39 (39%) of dengue patients versus 14/49 (29%) of malaria patients; *p<0,0001*].

A statistically significant difference was found in respect to headache. Headache appeared most common in the dengue group, compared to the H1N1 and malaria groups. 26/39 (67%) of dengue patients presented with headache compared to 19/49 (39%) of malaria patients and 65/199 (33%) of H1N1 patients (*p<0,0001)*.

The distribution of sore throat in all three groups was very similar as well. 23/199 (12%) of H1N1 patients, 3/39 (8%) of dengue patients and 1/49 (2%) of malaria patients showed sore throat.

Figure 14 Patients with rhinitis, fatigue, headache and sore throat

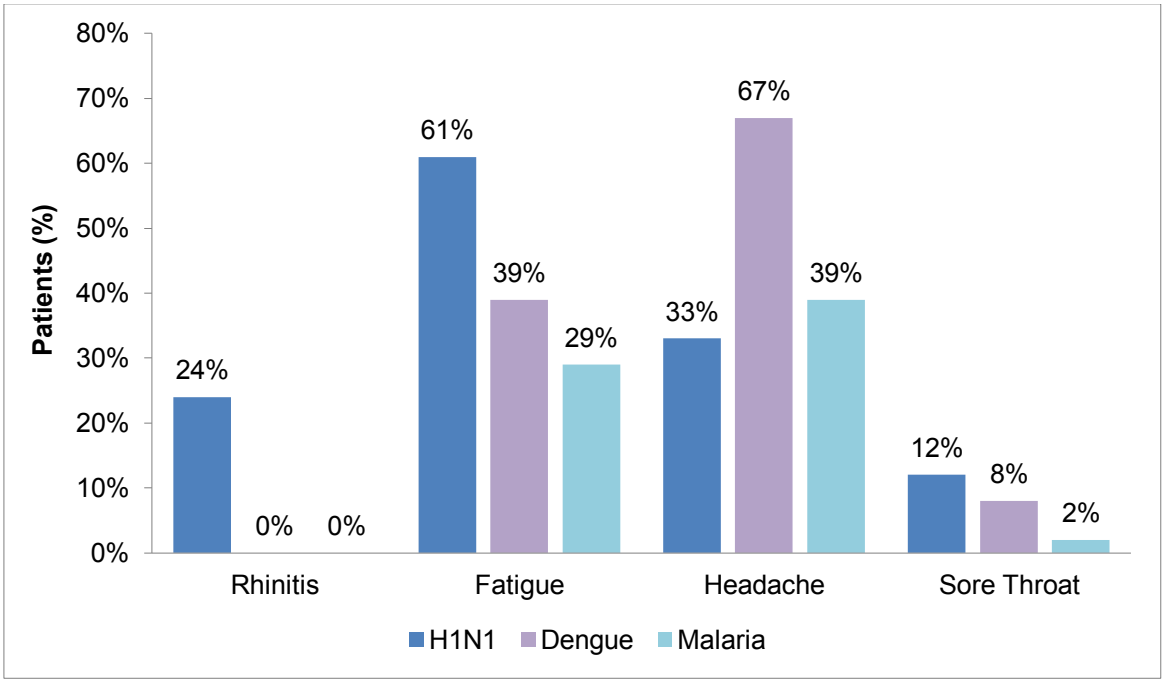


Table 5 Symptoms at first clinical presentation

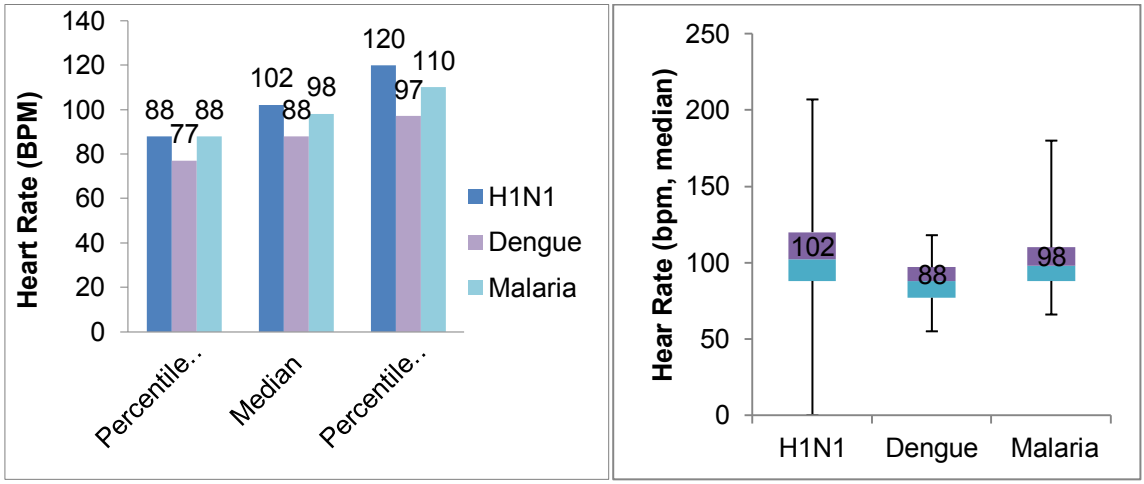
	H1N1 influenza n=199	Dengue n=39	Malaria n=49	p-value
Fever	185/199 (93%)	36/39 (93%)	39/48 (81%)	0,038
Rash	6/199 (3%)	14/39 (36%)	0/49 (0%)	<0,0001
Cough	146/199 (73%)	6/39 (15%)	5/49 (10%)	<0,0001
Dyspnea	43/199 (22%)	0/39 (0%)	2/49 (4%)	<0,0001
Thoracic Pain	17/199 (9%)	0/39 (0%)	2/49 (4%)	
Diarrhea	21/199 (11%)	17/39 (44%)	5/49 (10%)	<0,0001
Nausea/Vomiting	49/199 (25%)	10/39 (26%)	18/49 (37%)	
Abdominal Pain	12/199 (6%)	9/39 (23%)	3/49 (6%)	<0,008
Myalgia	48/199 (24%)	19/39 (49%)	9/49 (18%)	<0,004
Rhinitis	48/199 (24%)	0/39 (0%)	0/49 (0%)	<0,0001
Fatigue	120/198 (61%)	15/39 (39%)	14/49 (29%)	<0,0001
Headache	65/199 (33%)	26/39 (67%)	19/49 (39%)	<0,0001
Sore Throat	23/199 (12%)	3/39 (8%)	1/49 (2%)	

5.6 Vital parameters

5.6.1 Heart Rate

The initial heart rate was significantly lower in the dengue group versus the H1N1 influenza group or the malaria group. The median heart rate in the dengue group was 88 (IQR 77-97) beats per minute (bpm) versus 102 (IQR 88-120) beats per minute in the H1N1 group and 98 (IQR 88-110) beats per minute in the malaria group *(p<0,0001)*. (see Figure 15).

Figure 15 Median Heart Rate (bpm) and Inter Quartile Range (percentile 25, percentile 75)

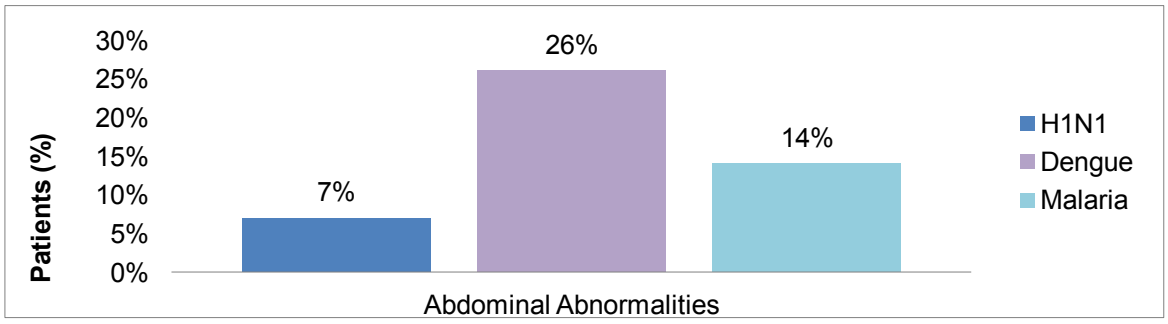


5.7 Physical examination

Only patients in the H1N1 group [28/199 (14%) of H1N1 patients versus 0/39 (0%) of dengue patients versus 0/49 (0%) of malaria patients; *p<0,001*] presented with signs of obstructive lung diseases, like wheezing.

A statistically significant difference regarding abdominal abnormalities was observed between the groups. The most pathologic abdominal findings were observed in patients of the dengue group [10/39 (26%)] compared to 14/199 (7%) of H1N1 patients and 7/49 (14%) of malaria patients *(p=0,005)*. (see Figure 16).

Figure 16 Pathological abdominal findings



Rales were reported in 19/199 (10%) of H1N1 compared to neither dengue nor malaria patients with no statistically significant difference.

There was also no observed significant difference in respect to conjunctivitis [8/199 (4%) of H1N1 patients versus 1/39 (3%) of dengue patients versus 2/49 (4%) of malaria patients]. Table 6 gives an overview about findings at physical examination.

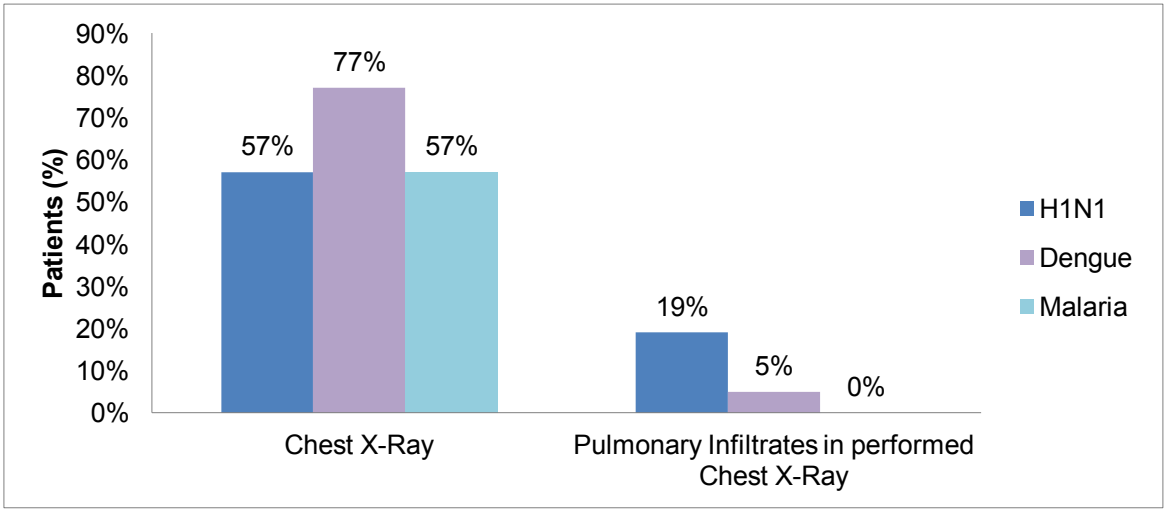
Table 6 Findings at physical examination

	H1N1 Influenza n=199	Dengue n=39	Malaria n=49	p-value
Wheezing	28/199 (14%)	0/39 (0%)	0/49 (0%)	<0,001
Abdominal abnormalities	14/199 (7%)	10/39 (26%)	7/49 (14%)	0,005
Rales	19/199 (10%)	0/39 (0%)	0/49 (0%)	
Conjunctivitis	8/199 (4%)	1/39 (3%)	2/49 (4%)	

5.8 Medical imaging (infiltrate in chest x-ray)

The frequency of ordered chest x-rays was almost equal in all three groups, with no significant difference observed, whereas a significant difference was observed concerning pulmonary infiltrates in the chest x-ray. 19% of performed chest x-rays in H1N1 patients and 5% of performed chest x-rays in dengue patients showed pulmonary infiltrates compared to none of the malaria patients *(p=0,001)*. (see Figure 17)

Figure 17 Performed chest X-Ray and pulmonary infiltrates

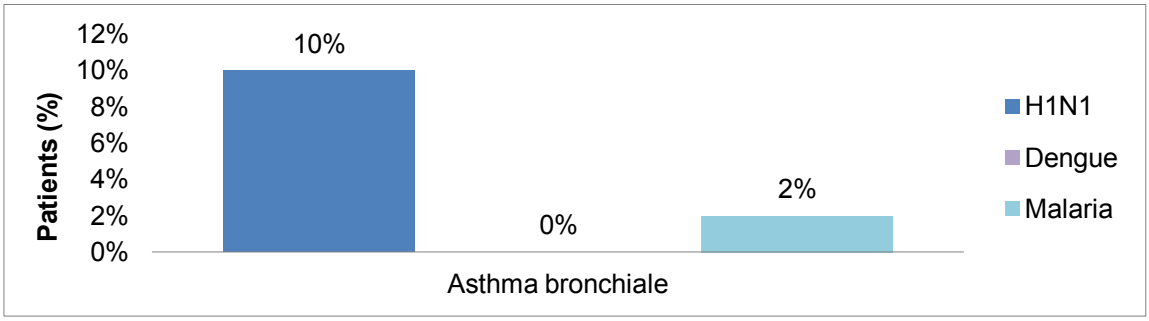


5.9 Underlying disease

5.9.1 Pulmonary Diseases

A significant difference was observed concerning Asthma bronchiale as an underlying disease in 20/199 (10%) of H1N1 patients, 1/49 (2%) of malaria patients and none of the dengue patients *(p=0,026).*

Figure 18 Patients with asthma bronchiale



No significant difference was found in respect to patients suffering from chronic obstructive pulmonary disease (COPD) [14/199 (7%) of H1N1 patients, 0/39 (0%) of dengue patients, 0/49 (0%) of malaria patients].

There was also no significant difference was observed regarding smoking in the medical history. 13/199 (7%) H1N1 patients, 4/39 (10%) dengue patients and 3/49 (6%) malaria patients admitted being smokers or having smoked in the past.

5.9.2 Cardiovascular Diseases

The amount of patients suffering from chronic coronary heart diseases was not significant in any of the three groups [H1N1 7/199 (4%); dengue 0/39 (0%); malaria 0/49 (0%)].

Also no significant difference was found concerning cardiac insufficiency as an underlying disease. Only H1N1 patients happened to suffer from cardiac insufficiency compared to the other two groups [H1N1 5/199 (3%), dengue 0/39 (0%), malaria 0/49 (0%)].

The number of patients with arterial hypertension was higher in the H1N1 group. 23/199 (12%) in this group suffered from arterial hypertension compared to 2/39 (5%) of dengue patients and 4/49 (8%) of malaria patients.

5.9.3 Neurologic disease

A significant difference was found in the number of patients with neurologic disease. 20/199 (10%) H1N1 patients had a history of neurologic disease compared to either dengue or malaria patients with none (*p=0,009)*.

5.9.4 Other Diseases (Renal insufficiency, Liver disease, Diabetes Mellitus, Malignancies)

No significant differences between the three groups were found concerning the following underlying diseases:

Renal insufficiency: 8/199 (4%) of H1N1 patients versus 0/39 (0%) of dengue patients versus 0/49 (0%) of malaria patients.

Liver disease: 6/199 (3%) of H1N1 patients versus 2/39 (5%) of dengue patients versus 0/49 (0%) of malaria patients.

Diabetes mellitus: 10/199 (5%) of H1N1 patients versus 0/39 (0%) of dengue patients versus 1/49 (2%) of malaria patients.

Malignancies: 10/199 (5%) of H1N1 patients versus 0/39 (0%) of dengue patients versus 1/49 (2%) of malaria patients.

5.9.5 Pregnancy

In the H1N1 group there were 8/199 (4%) pregnant patients during the survey. There was only 1/49 (2%) cases of patient pregnancy in the malaria group and in the dengue group there were no cases of patient pregnancy in the past medical history.

Table 7 is a summary of underlying diseases in H1N1, dengue and malaria patients.

Table 7 List of underlying diseases in H1N1, dengue and malaria patients

	H1N1 Influenza n=199	Dengue n=39	Malaria n=49	p-value, if significant
Asthma bronchiale	20 (10%)	0 (0%)	1 (2%)	0,026
COPD	14 (7%)	0 (0%)	0 (0%)	
Smoker	13 (7%)	4 (10%)	3 (6%)	
Coronary heart disease	7 (4%)	0 (0%)	0 (0%)	
Cardiac insufficiency	5 (3%)	0 (0%)	0 (0%)	
Arterial hypertension	23 (12%)	2 (5%)	4 (8%)	
Renal insufficiency	8 (4%)	0 (0%)	0 (0%)	
Liver disease	6 (3%)	2 (5%)	0 (0%)	
Diabetes mellitus	10 (5%)	0 (0%)	1 (2%)	
Malignancies	10 (5%)	0 (0%)	1 (2%)	
Neurologic disease	20 (10%)	0 (0%)	0 (0%)	0,009
Pregnant	8 (4%)	0 (0%)	1 (2%)	

5.10 Laboratory parameters

The following laboratory parameters were reviewed via the MEDOCS System and were subject to availability, meaning the amount of available data may vary from parameter to parameter and time of admission.

Table 8 and Table 9 give an overview of all the laboratory values at the admission date and in a five-day course observed.

Table 8 Laboratory admission parameters at outpatient clinic/ ER (=day 1) of patients with H1N1, Dengue and Malaria (median and IQR displayed)

	H1N1	Dengue	Malaria	p-value, if significant
Blood Count				
WBC (G/l)	6,74 (IQR 5-8,61)	5,11 (IQR 3,06-7,46)	5,86 (IQR 4,52-6,94)	0,002
Hemoglobin (g/dl)	13,5 (IQR 12,65-14,70)	14,2 (IQR 12,9-15)	13,8 (IQR 12,15-14,7)	
Hematocrit (%)	39,6 (IQR 36,78-43)	40,9 (IQR 38-43)	40 (IQR 35-43)	
Thrombocytes (G/l)	204 (IQR 166-243)	172 (IQR 118-229)	92 (IQR 65,00-115)	0,001
Neutrophils (%)	73 (IQR 62-81)	65 (IQR 56-78)	71 (IQR 62-79)	
Eosinophils (%)	0 (IQR 0-1)	0 (IQR 0-1)	0,05 (IQR 0-1)	
Monocytes (%)	9 (IQR 6-12)	10 (IQR 6 -12)	8 (IQR 6-11)	
Lymphocytes (%)	16 (IQR 10-25)	21 (IQR 14-30)	19,5 (IQR 11-27)	
Clinical chemistry				
C-reactive protein (mg/L)	17,4 (IQR 5,5-43,5)	13 (IQR 3-36,2)	98,7 (IQR 55,95-152,5)	<0,0001
Creatinekinase (U/l)	127 (IQR 68-194)	87 (IQR 67-121)	74 (IQR 45-158)	
Bilirubin (total; mg/dl)	0,4 (IQR 0,29-0,67)	0,43 (IQR 0,36-0,62)	1,24 (IQR 0,93-2,34)	<0,0001
Creatinine (mg/dl)	0,87 (IQR 0,63-1,18)	0,95 (IQR 0,81-1,06)	1,01 (IQR 0,84-1,14)	
Gamma GT (U/l)	25 (IQR 15-49)	46,5 (IQR 27-84)	72 (IQR 34-140)	<0,0001
AST (U/l)	29 (IQR 24-43)	48,5 (IQR 30-87)	44 (IQR 33–63)	<0,0001
ALT (U/l)	20 (IQR 15-34)	43,5 (IQR 28-93)	44 (IQR 25-70)	<0,0001
LDH (U/l)	223 (IQR 178-270)	267 (IQR 204-394)	305,5 (IQR 243-414)	<0,0001

Table 9 Laboratory parameters of patients with H1N1, Dengue and Malaria during hospitalization (median and IQR displayed)

	H1N1	Dengue	Malaria	p-value, if significant
Days 2 or 3				
Blood Count				
WBC (G/l)	4,88 (IQR 3,90-7,07)	4,03 (IQR 2,69-6,57)	4,17 IQR 3,39-5,9)	
Thrombocytes (G/l)	181 (IQR 140-213)	160 (IQR 60 -202)	71 (IQR 49-112)	<0,0001
Neutrophils (%)	64 (IQR 45-75)	56 (IQR 41-76)	60 (IQR 46-68)	
Eosinophils (%)	0 (IQR 0-1)	1 (IQR 0-2)	1 (IQR 0-2)	<0,0001
Monocytes (%)	10 (IQR 7-13)	11 (IQR 5-14)	12,5 (IQR 7-16)	
Lymphocytes (%)	24 (IQR 15-39)	26 (IQR 15-40)	28 (IQR 18-35)	
Clinical chemistry				
C-reactive protein (mg/l)	32 (IQR 10,8-67)	26,8 (IQR 11,50-97,25)	142 (IQR 84,1-187)	<0,0001
Days 4 or 5				
Blood Count				
WBC (G/l)	5,09 (IQR 3,77-7,44)	4,57 (IQR 3,26-5,81)	4,87 (IQR 4,1-7,89)	
Thrombocytes (G/l)	194 (IQR 121-234)	148 (IQR 114-202)	109 (IQR 73-155)	0,001
Neutrophils (%)	60 (IQR 43-79)	47 (IQR 36-63)	48 (IQR 37-58)	0,007
Eosinophils (%)	0,15 (IQR 0-2)	2 (IQR 1-3)	1 (IQR 1-3)	0,002
Monocytes (%)	9 (IQR 7-13)	11 (IQR 8-20)	11 (IQR 7-14)	
Lymphocytes (%)	27,50 (IQR 13-40)	30 (IQR 25-42)	37 (IQR 29-47)	0,025
Clinical chemistry				
C-reactive protein (mg/l)	22,65 (IQR 10-66)	6,35 (IQR 3,83-39,6)	80 (IQR 46,5-100,5)	<0,0001

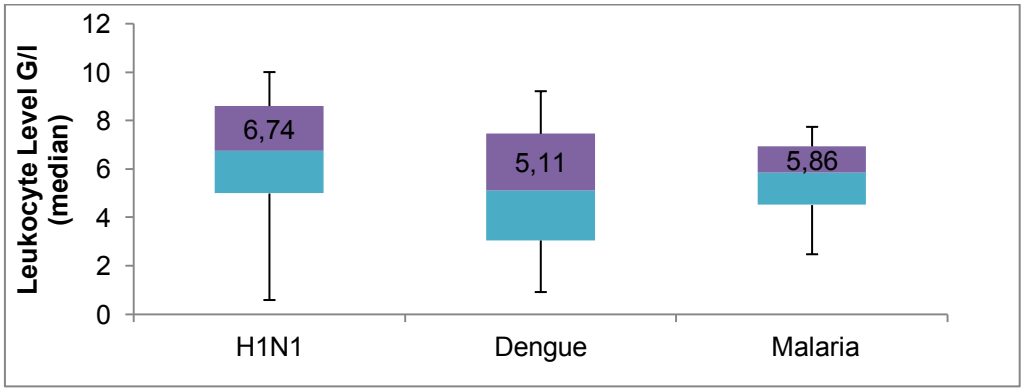
5.10.1 Blood count

5.10.1.1 White blood cells

5.10.1.1.1 White blood cells at presentation

At time of admission we observed a normal leukocyte range (median, reference range 4,4-11,3 G/l) in all three groups and found a statistically significant difference concerning leukocyte level at day of admission. The H1N1 group (n=194) presented with a median range of 6,74 G/l (IQR 5-8,61) compared to the dengue group (n=39) with 5,11 G/l (IQR 3,06-7,46) and the malaria group (n=48) with 5,86 G/l (IQR 4,52-6,94) (*p=0,002*).

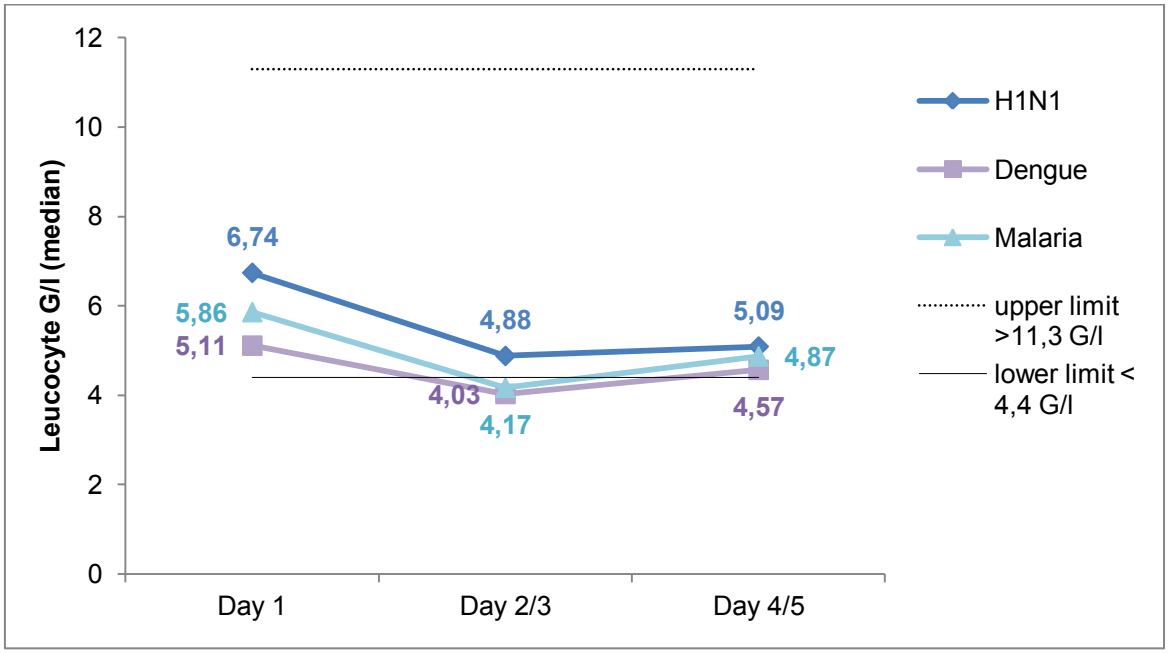
Figure 19 Leukocytes level at day of admission, showing median (and IQR)



5.10.1.1.2 White blood cell development in the first five days

Regarding the leukocyte level in a five day (day 2/3 and day 4/5) course, a significant difference wasn't observed, and leukopenia appeared in the dengue and malaria group on day 2/3. On day 2/3 patients in the H1N1 group (n=98) had a median range of 4,88 G/l (IQR 3,90-7,07), the dengue group (n=23) 4,03 G/l (IQR 2,69-6,57) and the malaria group (n=43) 4,17 G/l IQR 3,39-5,9) in leukocyte levels. The median range of leukocyte level was 5,09 G/l (IQR 3,77-7,44) in the H1N1 group (n=56), 4,57 G/l (IQR 3,26-5,81) in the dengue group (n=28) and 4,87 G/l (IQR 4,1-7,89) in the malaria group (n=34) on day 4/5 of admission.

Figure 20 Leukocyte levels on day 1, 2/3 and 4/5 in H1N1, Dengue and Malaria (median)

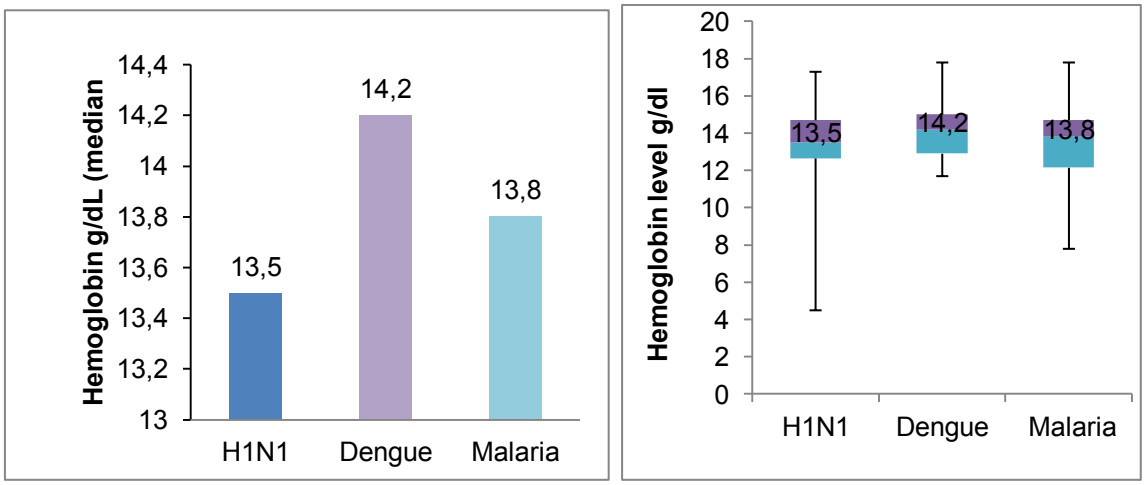


5.10.1.2 Hemoglobin/ Hematocrit

5.10.1.2.1 Hemoglobin at presentation

In respect of the hemoglobin concentration, the levels in all three groups were in a normal range (reference range 12-15,3 g/dl) and no significant difference was detected. The median hemoglobin concentration was 13,5 g/dl (IQR 12,65-14,70) in the H1N1 group (n=185), 14,2 g/dl (IQR 12,9-15) in the dengue group (n=39) and 13,8 g/dl (IQR 12,15-14,7) in the malaria group (n=49).

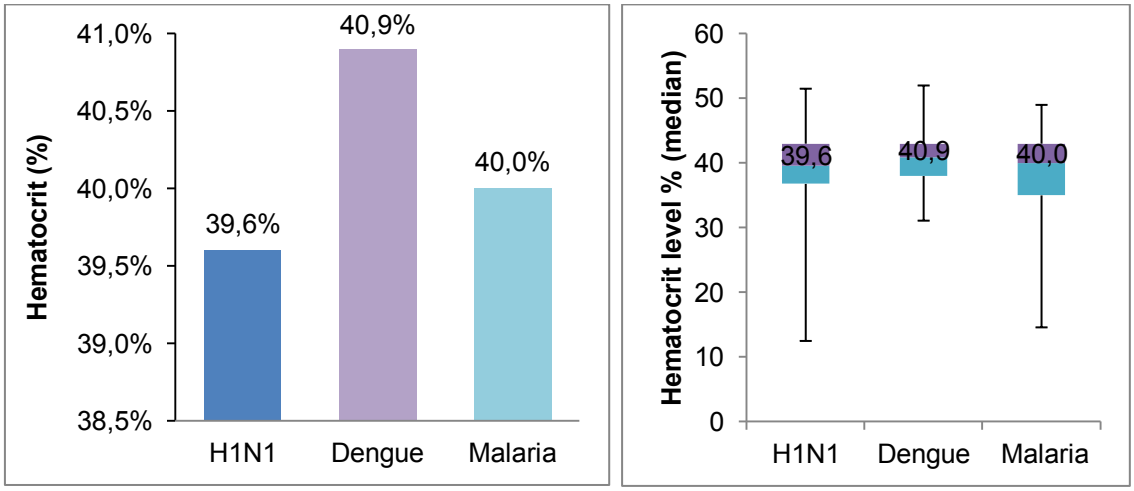
Figure 21 Hemoglobin level at presentation (median)



5.10.1.2.2 Hematocrit at presentation

The median hematocrit was also observed to be in a normal range (reference range 35,0-45,0%) with no statistical difference. In the H1N1 group (n=178) the median hematocrit ratio was 39,6% (IQR 36,78-43), in the dengue group (n=39) 40,9% (IQR 38-43) and in the malaria group (n=49) 40% (IQR 35-43).

Figure 22 Hematocrit level at presentation (median)



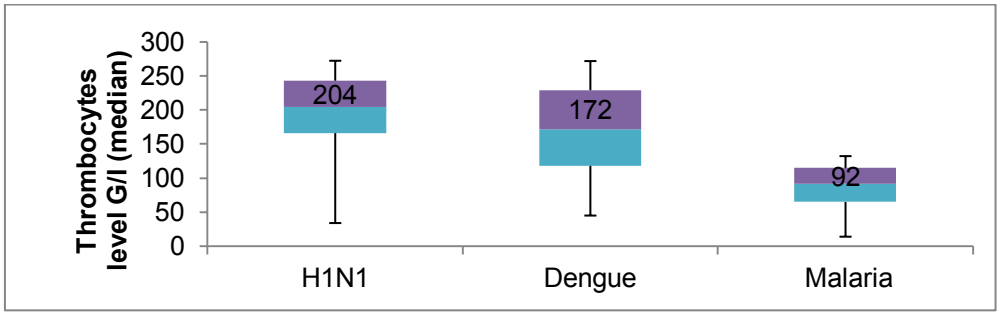
5.10.1.3 Thrombocytes

In general the median thrombocyte concentration was in a normal range within the H1N1 and dengue groups [but always in the lower third (140-240 G/l) of the normal range (140-440 G/l)], thrombocytopenia (thrombocyte level <140 G/l) was only recognized in the malaria group within the whole five-day course.

5.10.1.3.1 Thrombocytes at presentation

As mentioned above, thrombocytopenia (depending on the median thrombocyte concentration) occurred only in the malaria group, normal thrombocyte concentration was found in the H1N1 and dengue groups on the first day of admission. A statistically significant difference was observed between the malaria group, the H1N1 and the dengue groups at presentation. The median range of thrombocytes in the malaria group (n= 48) on the first day was 92 G/l (IQR 65,00-115) compared to a 204 G/l (IQR 166-243) thrombocyte level in the H1N1 group (n= 182) and to 172 G/l (IQR 118-229) in the dengue group (n= 39) *(p<0,0001)*.

Figure 23 Thrombocytes level at day of admission, showing median (and IQR)



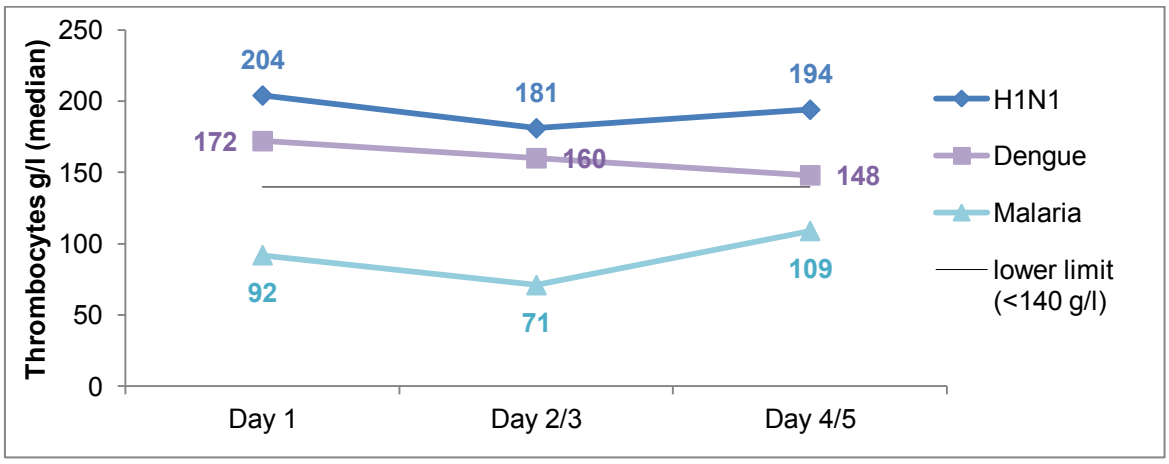
5.10.1.3.2 Thrombocytes development in the first five days

We observed a significant difference in the thrombocyte levels, not only on the first day but also within in the five-day course.

Regarding the median range of thrombocytes on day 2/3, the level was 71 G/l (IQR 49-112) in the malaria group (n= 43), 181 G/l (IQR 140-213) in the H1N1 group (n= 97) and 160 G/l (IQR 60 -202) in the dengue group (n= 23) (*p<0,0001)*.

On day 4/5 the median thrombocyte level was 109 G/l (IQR 73-155) in the malaria group compared (n= 33) to 194 G/l (IQR 121-234) in the H1N1 group (n= 57) and to 148 G/l (IQR 114-202) in the dengue group (n= 28) *(p=0,001)*.

Figure 24 Thrombocyte levels on day 1, 2/3, 4/5 in H1N1, Malaria and Dengue (median)

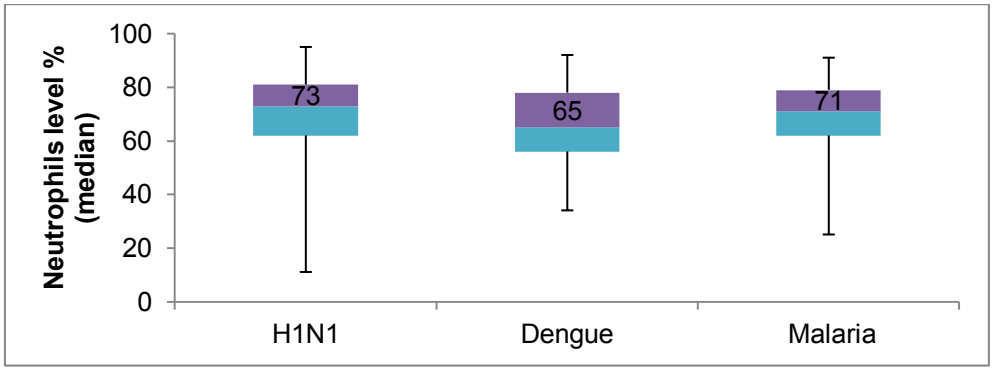


5.10.1.4 Neutrophils

5.10.1.4.1 Neutrophils at presentation

Neutrophil levels were within the normal range (reference range 50-75%) in all three groups, no significant difference was detected. The median neutrophils range in the H1N1 group (n= 167) was 73% (IQR 62-81), 65% (IQR 56-78) in the dengue group (n= 39) and 71% (IQR 62-79) in the malaria group (n= 47).

Figure 25 Neutrophils level at day of admission, showing median (and IQR)

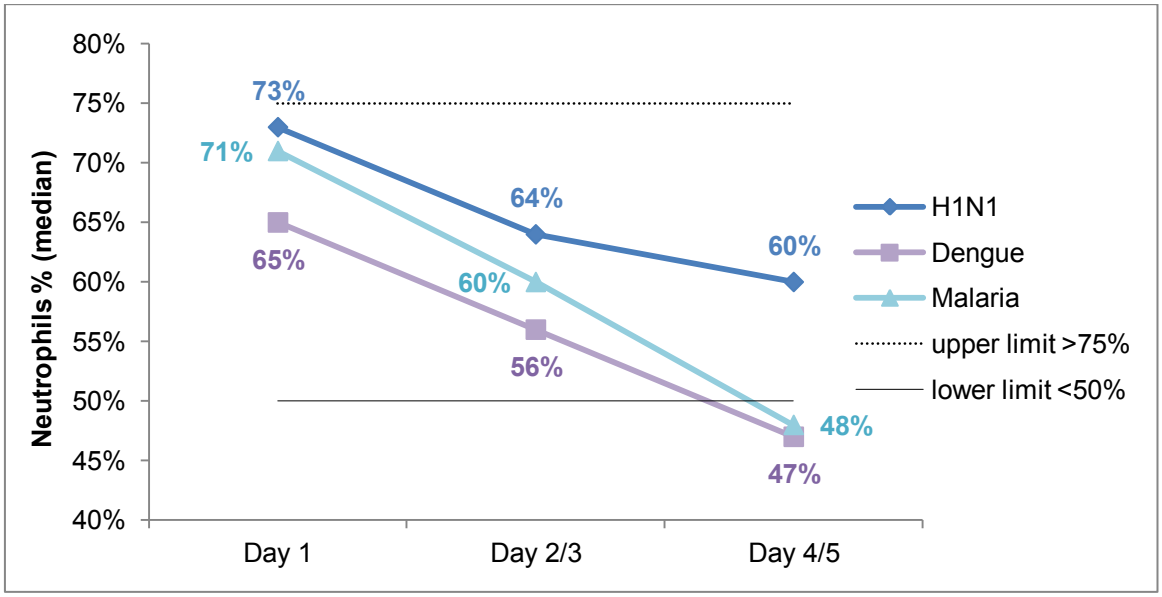


5.10.1.4.2 Neutrophils development in the first five days

On day 2/3 of admission the median neutrophil concentration was within the reference range in all three groups [64% (IQR 45-75) H1N1 group (n= 90); 56% (IQR 41-76) dengue group (n= 22); 60% (IQR 46-68) malaria group (n= 42)].

The median neutrophil counts on day 4/5 were slightly decreased within the dengue and malaria groups compared to the H1N1 group, with a significant difference. In the H1N1 group (n= 48) the neutrophil count was 60% (IQR 46-68) compared to the dengue group (n=28) with a median neutrophil count of 47% (IQR 36-63) and 48% (IQR 37-58) in the malaria group (n= 33) (*p=0,007*).

Figure 26 Median neutrophils count on day 1, 2/3 and 4/5 of admission in H1N1, Dengue and Malaria patients



5.10.1.5 Eosinophils

5.10.1.5.1 Eosinophils at presentation

During the whole five-day course all eosinophil levels were within the reference rage of 0-5% in all groups. Determining the median eosinophil counts, no significant difference was observed on day 1 of admission. The median eosinophil range on the first day of admission was 0% (IQR 0-1) in the H1N1 group (n=166), 0% (IQR 0-1) in the dengue group (n=39) and 0,05% (IQR 0-1) in the malaria group (n=48).

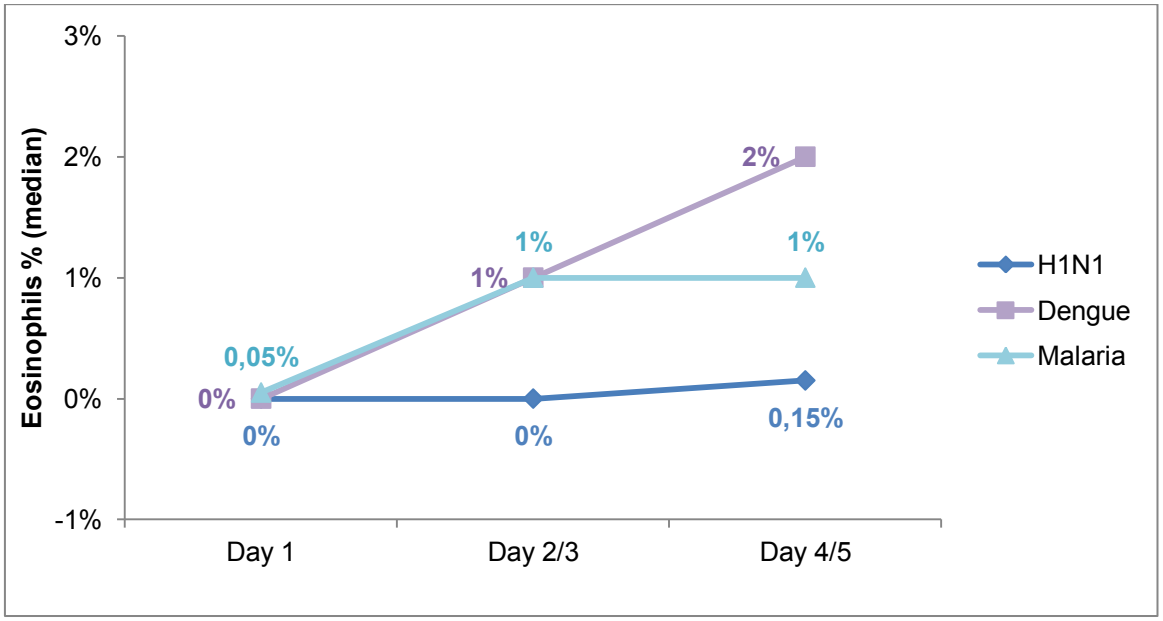
5.10.1.5.2 Eosinophils development in the first five days

Regarding the five-day course of median eosinophil counts we observed a statistically significant difference on day 2/3 and day 4/5 as well.

The median eosinophil range on day 2/3 was 0% (IQR 0-1) in the H1N1 group (n=89), 1% (IQR 0-2) in the dengue group (n=21) and 1% (IQR 0-2) in the malaria group (n=43) with a significance of *p<0,0001*.

The H1N1 group (n=49) had a median eosinophil count of 0,15% (IQR 0-2) on day 4/5 compared to a count of 2% (IQR 1-3) in the dengue group (n=28) and 1% (IQR 1-3) in the malaria group (n=33) with a significance of *p=0,002*.

Figure 27 Median eosinophils count on day 1, 2/3 and 4/5 of admission in H1N1, Dengue and Malaria patients



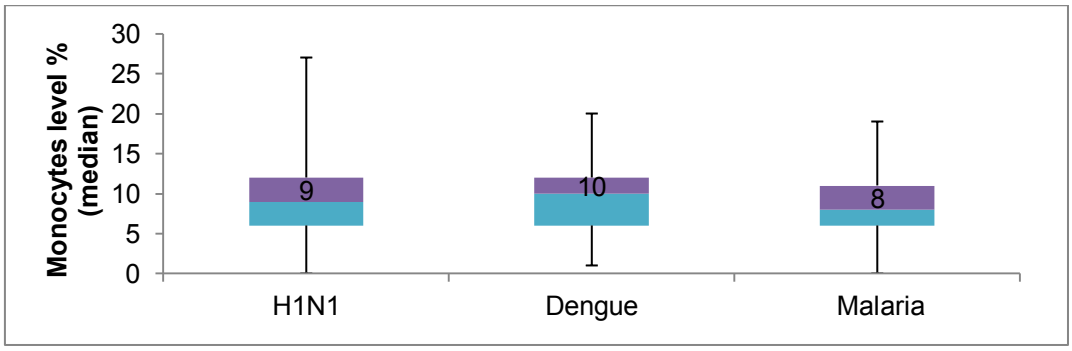
5.10.1.6 Monocytes

Mainly the median monocyte levels were within the normal range on each day of surveillance with a reference range of 2-12%. No significant difference was observed in any group during the five-day course.

5.10.1.6.1 Monocytes at presentation

The median monocyte count on day of admission was normal in all groups, at 9% (IQR 6-12) in the H1N1 group (n=167), 10% (IQR 6-12) in the dengue group (n=39) and 8% (IQR 6-11) in the malaria group (n=48).

Figure 28 Monocytes level at day of admission, showing median (and IQR)

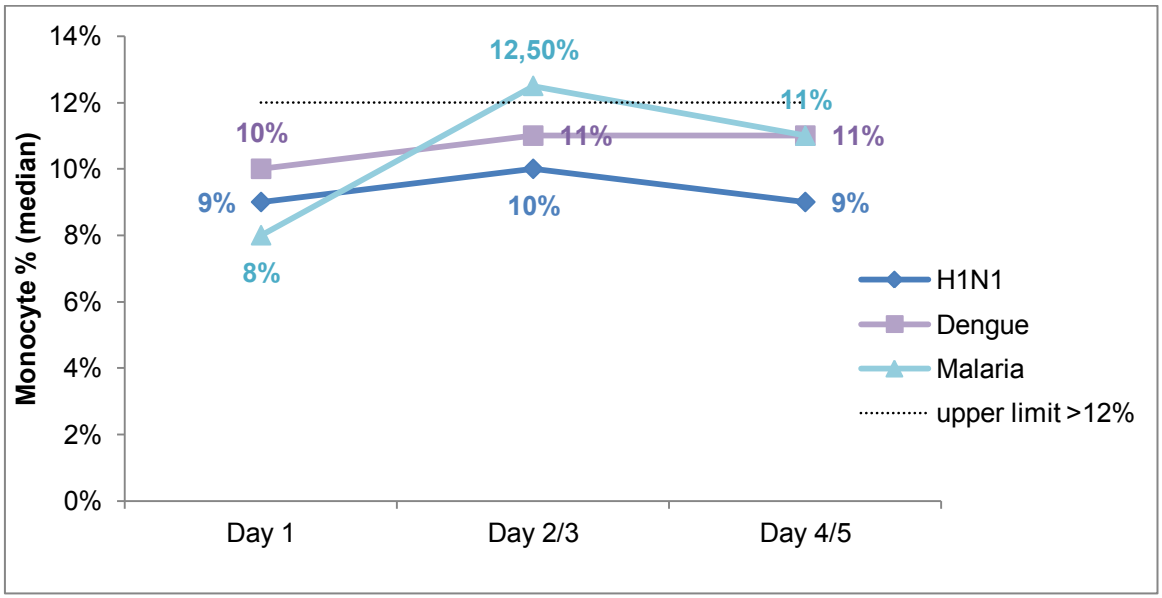


5.10.1.6.2 Monocytes development in the first five days

On day 2/3 the median monocyte level was slightly increased in the malaria group [10% (IQR 7-13) in the H1N1 group (n=90), 11% (IQR 5-14) in the dengue group (n=22) and 12,5% (IQR 7-16) in the malaria group (n=42)].

The median monocyte count was also within the normal range in all three groups on day 4/5 of admission with a median count of 9% (IQR 7-13) in the H1N1 group (n=49), 11% (IQR 8-20) in the dengue group (n=28) and 11% (IQR 7-14) in the malaria group (n=33).

Figure 29 Monocytes count on day 1, 2/3, and 4/5 in the H1N1, Dengue and Malaria group (median)



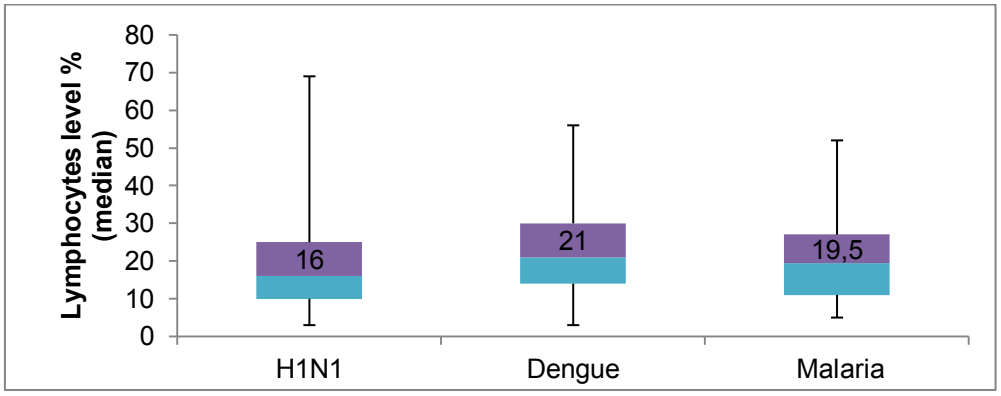
5.10.1.7 Lymphocytes

5.10.1.7.1 Lymphocytes at presentation

At the first day of admission lymphocytes were observed to be slightly decreased within the H1N1 group and malaria group compared to the dengue group regarding a reference range of 20-40%.

The median lymphocyte range was 16% (IQR 10-25) in the H1N1 group (n=173), 21% (IQR 14-30) in the dengue group (n=39) and 19,5% (IQR 11-27) in the malaria group (n=48) with no significant difference detected.

Figure 30 Lymphocytes level at day of admission, showing median (and IQR)

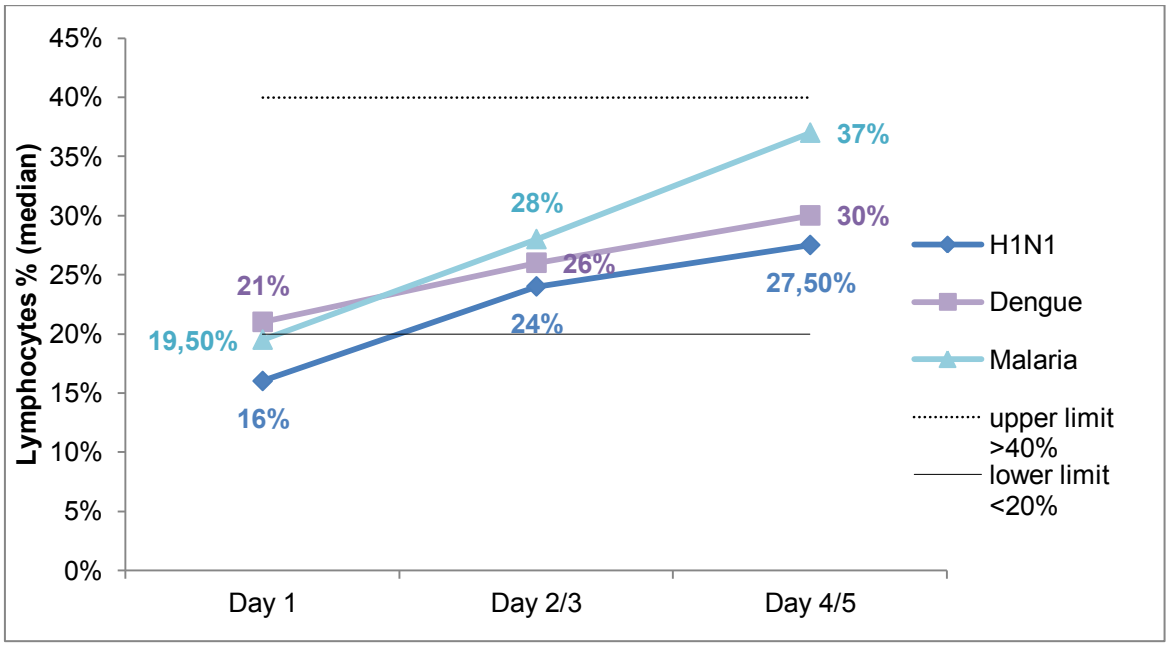


5.10.1.7.2 Lymphocytes development in the first five days

On day 2/3 of admission the lymphocyte count was within the reference range in all three groups. No significant difference was observed concerning a median lymphocyte count of 24% (IQR 15-39) in the H1N1 group (n=90), 26% (IQR 15-40) in the dengue group (n=22) and 28% (IQR 18-35) in the malaria group (n=43).

A statistically significant difference (*p=0,025*) was observed regarding the median lymphocyte range on day 4/5 in the malaria group (n=33) with a count of 37% (IQR 29-47) compared to the H1N1 group (n=48) with a count of 27,5% (IQR 13-40) and the dengue group (n=33) with a count of 30% (IQR 25-42).

Figure 31 Lymphocytes count on day 1, 2/3 and 4/5 of admission in the H1N1, Dengue and Malaria group (median)



5.10.2 Clinical Chemistry

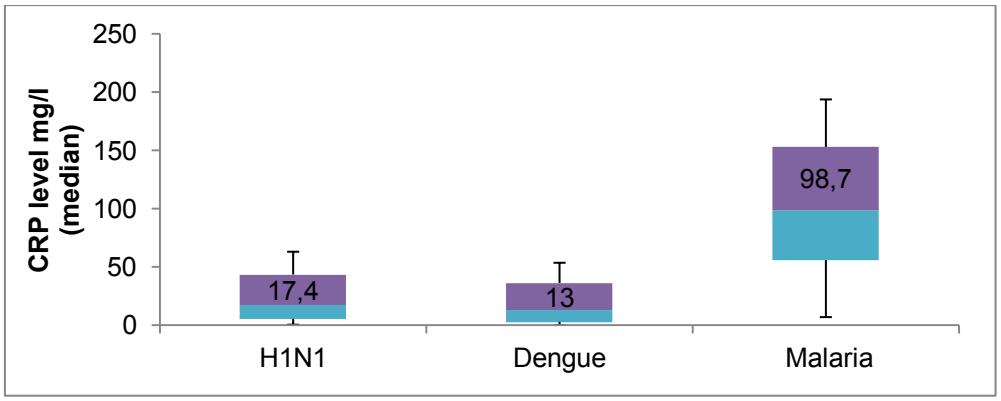
5.10.2.1 C-reactive protein (CRP)

The laboratory parameter C-reactive protein was observed to be significantly different on each day of surveillance within the three groups. CRP level was increased within all groups on all days, regarding the reference range of <5mg/l on.

5.10.2.1.1 CRP at presentation

The median CRP value on the first day of presentation was increased above reference range in all three groups with a statistically significant difference (*p<0,0001*) in the malaria group [98,7 mg/l (IQR 55,95-152,5); n=49] compared to the H1N1 group [17,4 mg/l (IQR 5,5-43,5); n=178] and the dengue group [13 mg/l (IQR 3 -36,2); n=39].

Figure 32 CRP level at day of admission, showing median (and IQR)



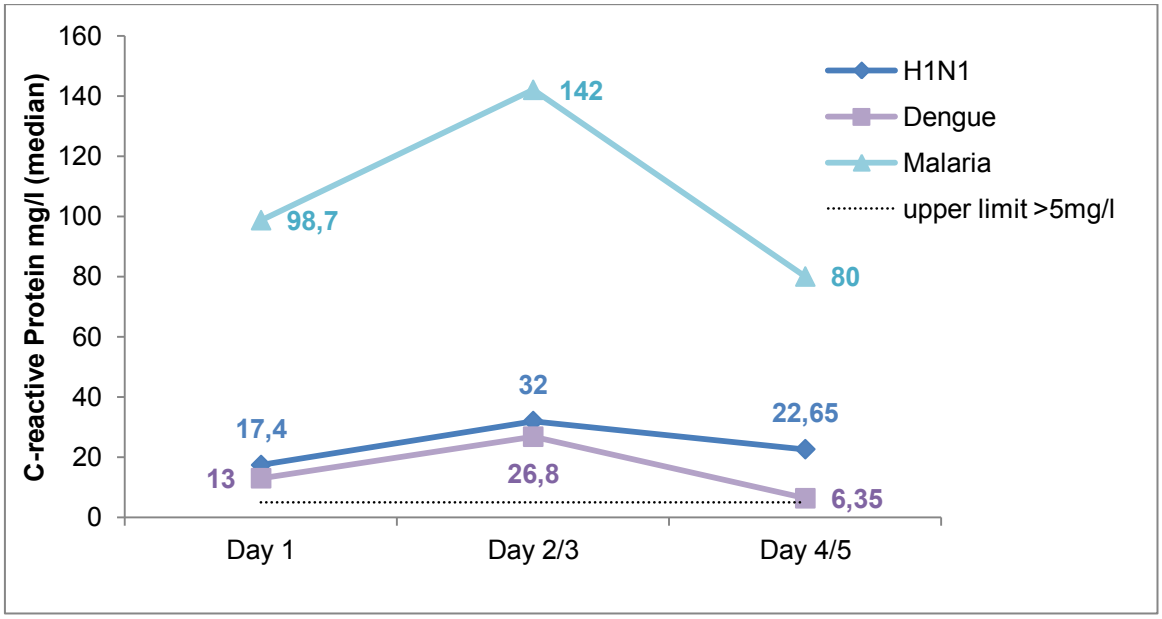
5.10.2.1.2 CRP development in the first five days

On days 2/3 and 4/5 as well, the median CRP value was always increased.

On day 2/3 we observed a significance of *p<0,0001* in the malaria group [142mg/l (IQR 84,1-187); n=33] compared to the H1N1 group [32 mg/l (IQR 10,8-67); n=95] and the dengue group [26,8 mg/l (IQR 11,5-97,25); n=18].

Also a significant difference was detected on day 4/5 in the malaria group [80 mg/l (IQR 47-101); n=13] compared to the H1N1 group [22,65 mg/l (IQR 10-66); n=54] and the dengue group [6,35 mg/l (IQR 3,83-39,6); n=26] (*p<0,0001*).

Figure 33CRP values on day 1, 2/3 and 4/5 of admission in the H1N1, Dengue and Malaria group (median)

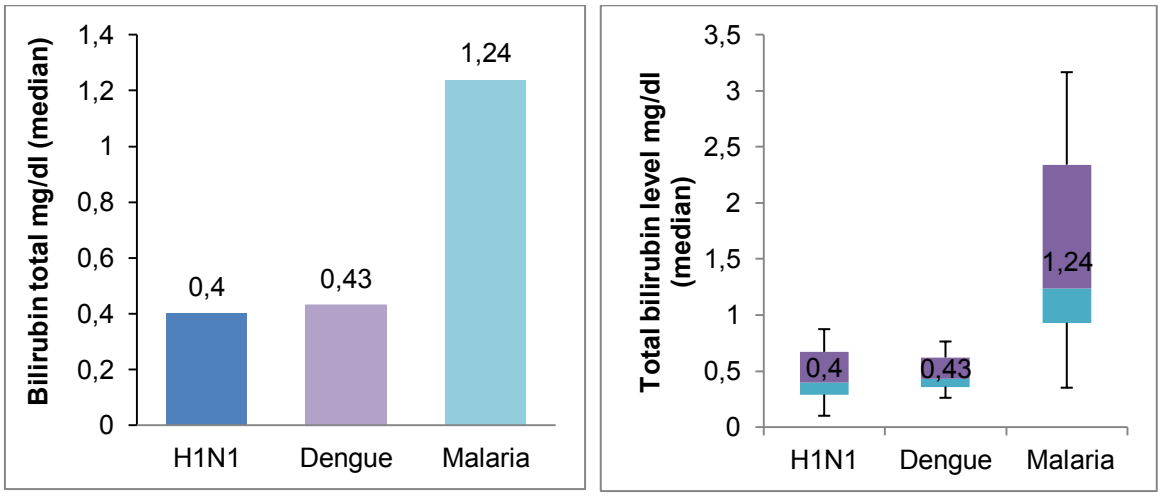


5.10.2.2 Bilirubin (total) at presentation

The median total bilirubin concentration was slightly increased in the malaria group compared to the H1N1 and dengue groups (reference range of 0,1-1,1 mg/dl), with a statistically significant difference of *p<0,0001.*

In the malaria group (n=43) the median bilirubin concentration was 1,24 mg/dl (IQR 0,93-2,34) compared to the H1N1 group (n=89) with a median concentration of 0,4 mg/dl (IQR 0,29-0,67) and the dengue group (n=29) with 0,43 mg/dl (IQR 0,36-0,62).

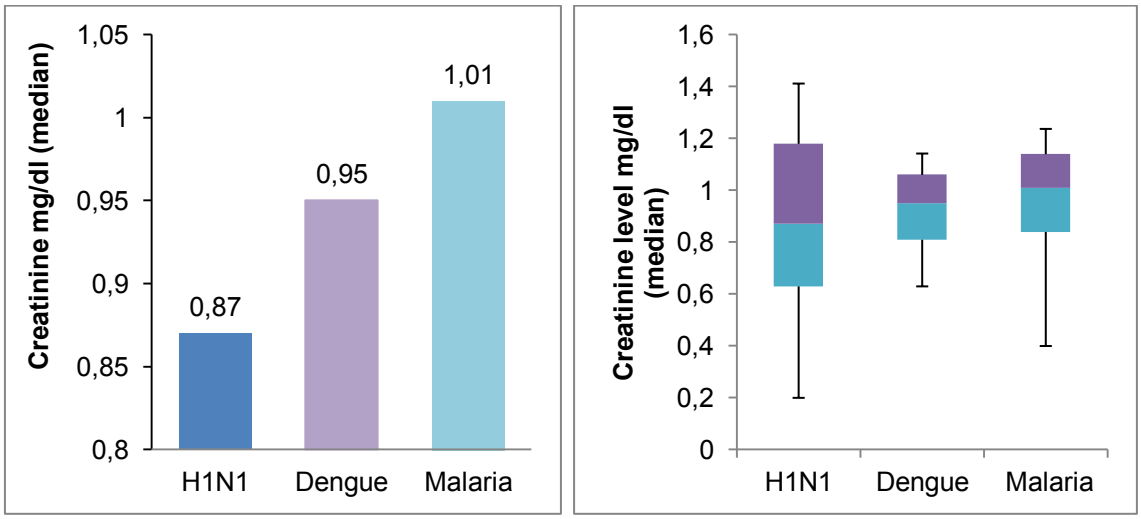
Figure 34 Total bilirubin concentration (median) at presentation



5.10.2.3 Creatinine at presentation

The median creatinine level was within a normal range in all three groups, concerning a reference range of 0,5-1,0 mg/dl, but with no significant difference. The median creatinine range was 0,87 mg/dl (IQR 0,63-1,18) in the H1N1 group (n=111), 0,95 mg/dl (IQR 0,81-1,06) in the dengue group (n=38) and 1,01 mg/dl (IQR 0,84-1,14) in the malaria group (n=49).

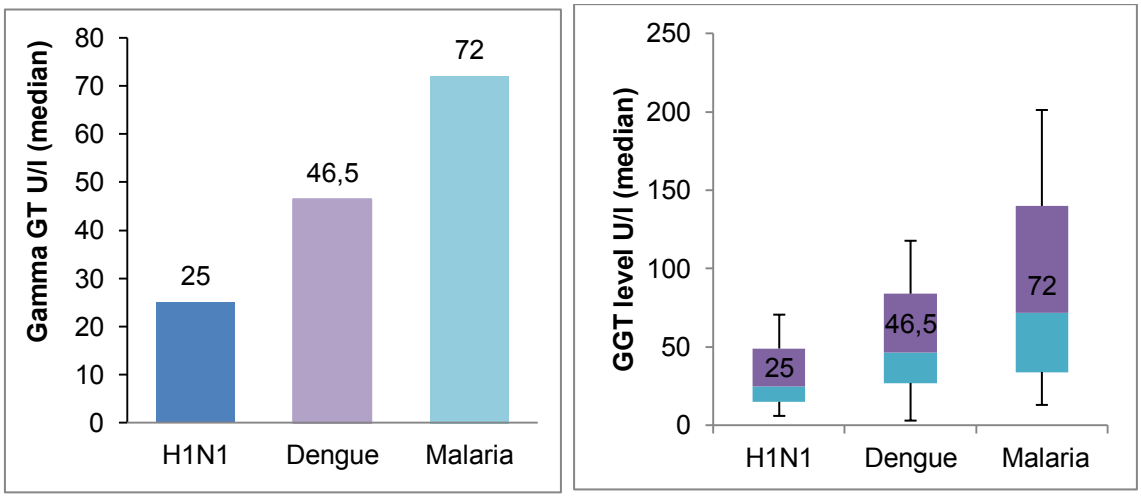
Figure 35 Creatinine on day 1 in the H1N1, Dengue and Malaria group (median)



5.10.2.4 Gamma-glutamyl transpeptidase (GGT) at presentation

Increased GGT levels were observed in the dengue and malaria groups with a reference range of 0-38 U/l, as well as a significant difference in all three groups. The median GGT range was 72 U/l (IQR 34-140) in the malaria group (n=49) compared to a range of 25 U/l (IQR 15-49) in the H1N1 group (n=108) and 46,5 U/l (IQR 27-84) in the dengue group (n=38) *(p<0,0001)*.

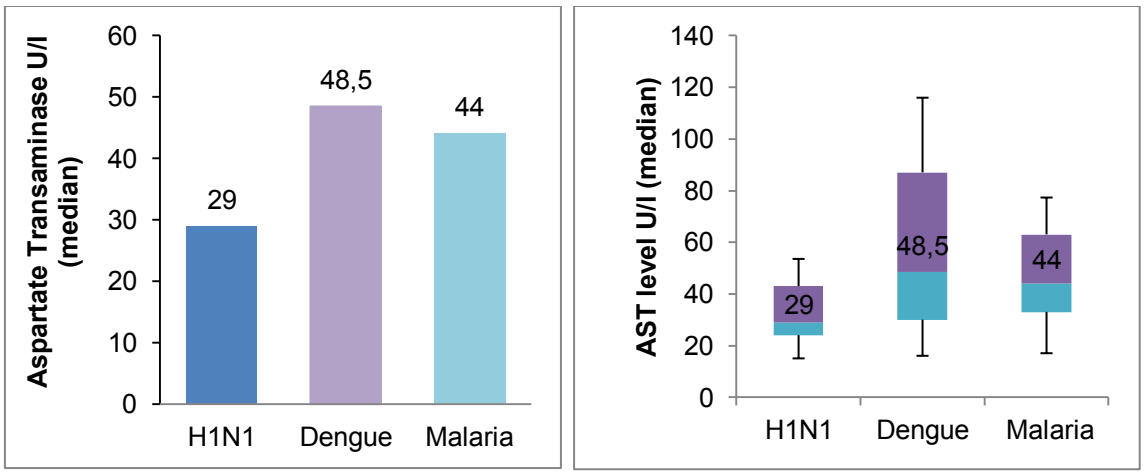
Figure 36 Gamma-glutamyl transpeptidase on day 1 in the H1N1, Dengue and Malaria group (median)



5.10.2.5 Aspartate Transaminase (AST) at presentation

Considering the median AST value on the first day, a significant difference of *p<0,0001* was observed concerning the H1N1 group compared to the dengue and the malaria groups. The median AST level was increased in the dengue and malaria groups (reference range <30 U/l) and was 29 U/l (IQR 24-43) in the H1N1 group (n=109) compared to 48,5 U/l (IQR 30-87) in the dengue group (n=38) and 44 U/l (IQR 33-63) in the malaria group (n=49).

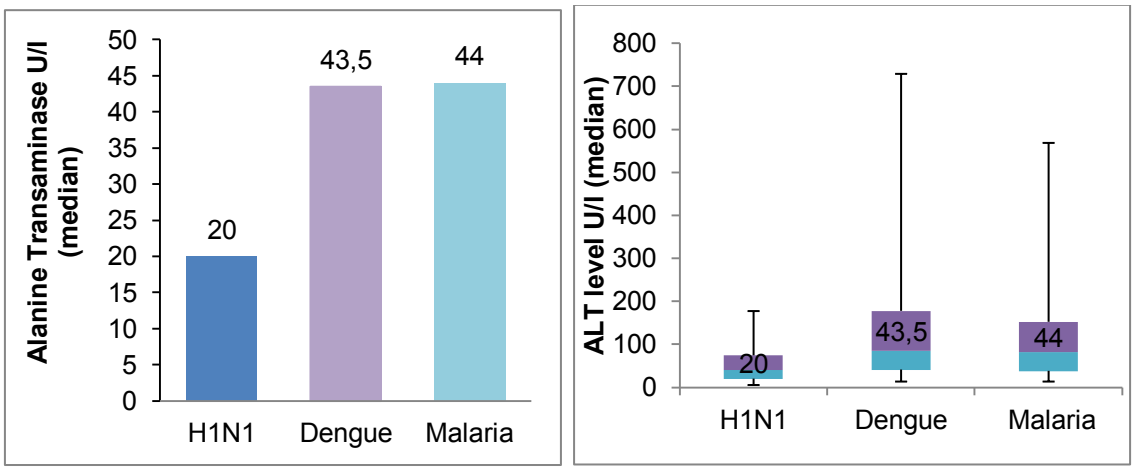
Figure 37 Aspartate Transaminase on day 1 in the H1N1, Dengue and Malaria group (median)



5.10.2.6 Alanine Transaminase (ALT) at presentation

A significant difference was observed in the median ALT concentration concerning the H1N1 group compared to the dengue and malaria groups. The ALT level was increased in the dengue and malaria group concerning a reference range of <35 U/l. The median ALT range was observed to be 20 U/l (IQR 15-34) in the H1N1 group (n=110) in contrast to 43,5 U/l (IQR 28-93) in the dengue group (n=38) and 44 U/l (IQR 25-70) in the malaria group (n=49) (*p<0,0001*).

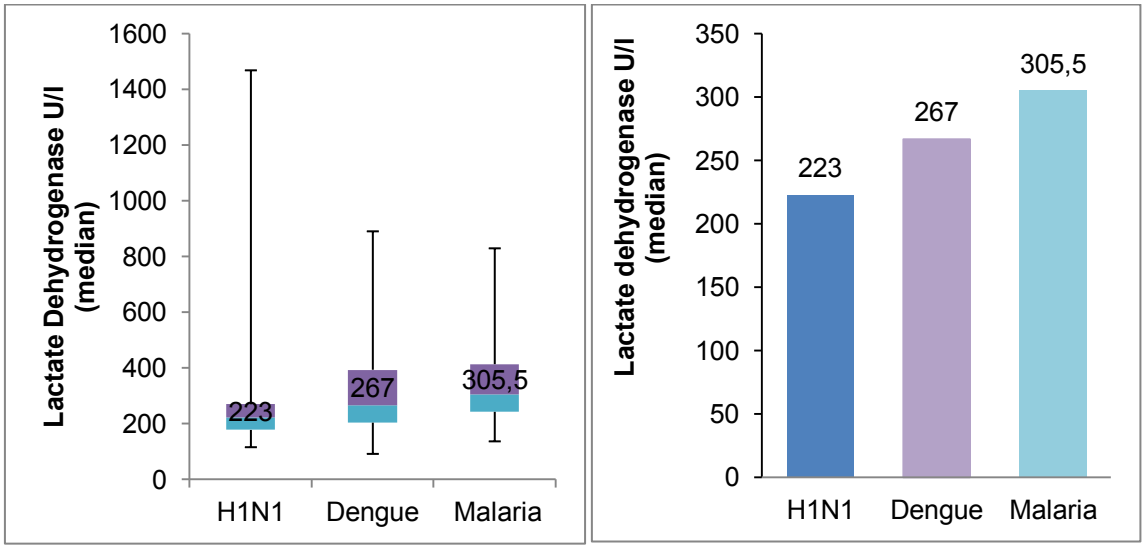
Figure 38 Alanine Transaminase on day 1 in the H1N1, Dengue and Malaria group (median)



5.10.2.7 Lactate dehydrogenase (LDH) at presentation

Considering the median LDH value, a statistically significant difference was also observed in the malaria group in contrast to the H1N1 and dengue groups. The median LDH concentration was within the reference range (120-240 U/l) in the H1N1 group, in the dengue and malaria groups the median value was slightly above reference range. The median concentration of LDH was 305,5 U/l (IQR 243-414) in the malaria group (n=48) compared to 223 U/l (IQR 178-270) in the H1N1 group (n=107) and 267 U/l (IQR 204-394) in the dengue group (n=37) (*p<0,0001*).

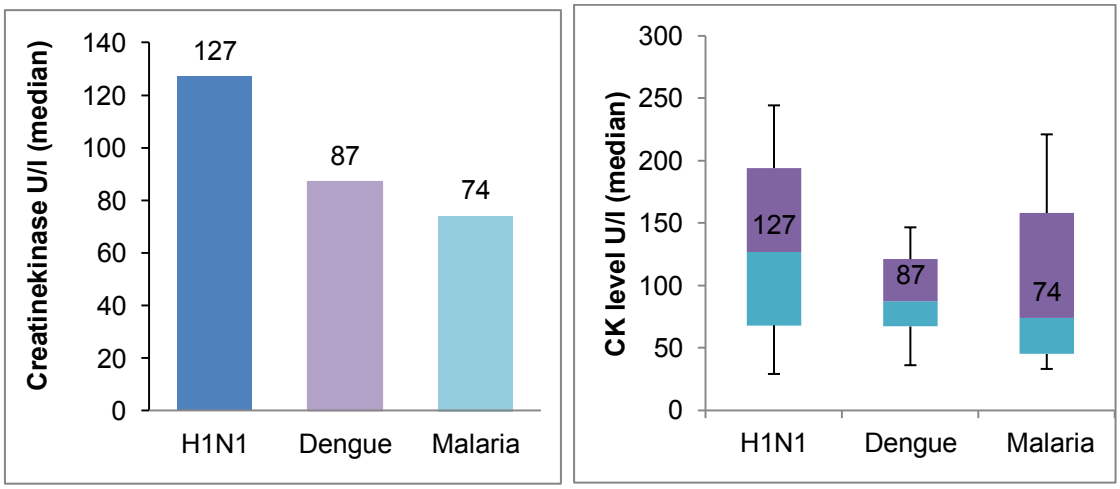
Figure 39 Median LDH level at presentation



5.10.2.8 Creatinekinase (CK) at presentation

Considering the Creatinekinase on the first day of admission, no significant difference was observed in the three groups and all CK levels were within the reference range of <145 U/l. The median CK concentration was 127 U/l (IQR 68-194) in the H1N1 group (n=82), 87 U/l (IQR 67-121) in the dengue group (n=18) and 74 U/l (IQR 45-158) in the malaria group (n=24)

Figure 40 Median creatinekinase level at presentation



6 Discussion

We retrospectively analyzed differences in demographic data; initial clinical presentation and laboratory parameters between patients with PCR confirmed H1N1 influenza, RDT confirmed dengue and patients with blood smear confirmed malaria infection. The aim of this study was to detect clinical and laboratory differences, specifically between H1N1 influenza and dengue disease, to facilitate early diagnosis and to provide the possibility of early and efficient targeted therapy (e.g. with neuraminidase inhibitors for influenza diseases).

As other studies showed, H1N1 is more likely to infect younger people or children (37-40). However, the age difference in our study is more related to the travel history (children are less likely to travel to tropical countries). A survey from the GeoSentinel Surveillance Network supports our conclusion that there is no difference in the age of returning travelers with fever (6).

In our survey 27% of H1N1 patients presented to emergency room only and 66% were admitted to normal wards, in contrast 74% of dengue patients and 90% of malaria patients were admitted to normal wards directly and only 6% of malaria patients were treated in the emergency room only. We believe, that returning travelers who present with fever are instantaneously admitted to normal wards, to find out the cause of fever (diagnostic tests like blood smear and dengue RDTs) and to provide proper treatment. The GeoSentinel Surveillance Network suggests that returning ill travelers consume sustainable medical care (6). Another explanation for the higher rate of exclusively ambulatory care in H1N1 patients, is that the time between the onset of disease and presentation at ER is quite short in H1N1 patients (1 day) compared to dengue and malaria patients (4 days), meaning H1N1 patients seek medical care immediately at onset of symptoms whereas dengue and malaria patients often need to return home first from their travels to be able seeking medical care. This coincides with the longer periods between onset of symptoms and presentation at hospital in dengue and malaria patients.

It is also interesting, that sudden onset was quite frequent in dengue and malaria cases and was significantly less frequent in H1N1 cases (82% of H1N1 patients vs. 95% of dengue patients and 96% of malaria patients, p=0,015). Accordingly, in our survey sudden onset is not a useful marker for H1N1 infections. Our findings correspond with those of a Mexican study where sudden onset occurred only in 75% of all H1N1 infections, which is fairly similar to our 82% (41).

We observed that H1N1 patients in general had more co-morbidities compared to dengue and malaria patients. This might be explained by the fact that co-morbid patients travel less and are in general missing in the returning traveler groups.

Pulmonary diseases such as asthma bronchiale (H1N1: 10%; dengue: 0%; malaria: 2%) and COPD, particularly affect H1N1 patients much more, due to influenza being a respiratory tract infection.

Fever is common in ill returning travelers and is one of the main reasons for hospitalization. In a recent report from GeoSentinel, the most common diagnoses in returning travelers with fever were malaria (21%) and dengue (6%, estimated higher rate due to often mild or nonspecific clinical manifestation), followed by febrile diarrheal disease (15%) and respiratory infections including influenza (14%) (6). In our cohort, fever was reported in 93% of H1N1 infection, 93% of dengue infection and 81% of malaria infections, but we believe that the lower rates in the malaria group were due to missing information and should range around 90% as well. Accordingly, we cannot use fever to differentiate H1N1, dengue and malaria from one another. Comparing our results with other studies we can see that the rate of fever is corresponding in the H1N1 group (12,42-45) and in the dengue group (46,47) and furthermore do they confirm our estimation, that malaria has also a higher rate of fever than in our cohort (6,48).

36% of our dengue patients reported rash compared to 3% of the H1N1 patients and 0% of the malaria patients (p<0,0001). So we may assess that appearance of rash or exanthema is relatively specific for dengue infection. A French study of 62 patients comparing chikungunya infection with dengue infection, observed that 26% of returning travelers presenting with febrile exanthema were diagnosed with dengue fever and conversely that out of 16 dengue cases, 81% presented with macular exanthema (49). The GeoSentinel Surveillance network analyzed dermatologic conditions in ill returning travelers, and observed that 3,4% of 4742

diagnoses were dengue infections, being one of the ten most frequent diagnoses with dermatologic manifestations (50).

Regarding our results concerning respiratory findings, we conclude that respiratory symptoms are specific for H1N1 influenza infections; again, considering the fact that H1N1 influenza is a respiratory tract infection. We observed an increased occurrence of coughing (73%), rhinitis (24%), wheezing (14%), dyspnea (22%), thoracic pain (9%) and infiltrates in chest x-ray (19%) in the H1N1 group compared to almost none of these symptoms in the dengue and malaria groups.

Patients who present with gastrointestinal symptoms like diarrhea, abdominal pain and abdominal findings in physical examination are more likely to suffer from dengue infection, rather than H1N1 influenza or malaria. Hence, abdominal manifestations especially diarrhea are relatively specific for dengue infection. In a study from Puerto Rico more than 50% of dengue patients suffered from nausea or vomiting and 30% from diarrhea (51). This result is in accordance with our findings; we observed that 44% of dengue patients suffered from diarrhea, compared to 11% of H1N1 patients and 10% of malaria patients. Furthermore, 23% of dengue patients reported abdominal pain. In a prospective observation study of early clinical and laboratory indicators of acute dengue illness in Thai children, 34% also reported abdominal pain (30). Severe abdominal pain is understood as a warning sign for sever dengue infection (24,52,53).

As many other studies showed, headache, myalgia and fatigue are non-specific symptoms (54-58). Although we calculated a significant difference concerning fatigue, myalgia and headache, we cannot use them as useful items for clinical differentiation due to low specificity of symptoms and relatively high appearance in all three groups (fatigue: 61% H1N1 vs. 39% dengue vs. 29% malaria; headache: 33% H1N1 vs. 67% dengue vs. 39% malaria; myalgia: 24% H1N1 vs. 49% dengue vs. 18% malaria).

Regarding our laboratory findings, we observed that thrombocyte levels were within a normal range in the H1N1 group (within the whole 5-day course) and we consider dengue or malaria infection as more likely, when the thrombocyte level is lower than 160 G/l. Conversely, if thrombocyte levels are above 120 G/l malaria infection is rather unlikely. This is coherent with a Belgian study, assessing that malaria is likely with the following features: enlarged spleen, thrombocytopenia (platelet count <150 G/l), and fever without localizing symptoms and

hyperbilirubinaemia (total bilirubin level >or=1.3 mg/dl; in our cohort total bilirubin level: 1,24 mg/dl) (59). Both Bottieau et al. and Kalayanarooj et al. published that skin rash, thrombocytopenia and leukopenia (leukocyte count <4,4 G/l) are predicted indicators for dengue infection, and furthermore do Kalayanarooj et al. suggest that serum AST levels are frequently elevated in DF (30,59), which was also the case in our dengue group (48,5 U/l on day 1). In contrast to a study from the United States of patients with 2009 H1N1 influenza, none of their laboratory findings confirm our results (60).

Another helpful tool to differentiate the three groups from one another would be the CRP level. Thanks to our results (H1N1: 17,4 mg/l; Dengue: 13 mg/l; Malaria: 98,7mg/dl on day 1) we can conclude that if CRP levels are above 50 mg/l on the day of admission, the diagnosis of malaria is more likely.

Our study has several limitations. Only a limited number of returning travelers with dengue and malaria disease presented to Styrian Hospitals, therefore our cohort was quite small. We included only patients with H1N1 infections and did not include other influenza serotypes, as PCR data was only available for H1N1 infections from the pandemic in 2009/2010. Data was only collected from patients that presented to Styrian hospitals only and had electronically available medical records; data of outpatients haven't been reviewed. Therefore, the data was complete in just 287/761 (38%) cases. Furthermore, patients were excluded from our study when their symptoms lasted for more than 7 days (onset of symptoms >7 days). Therefore, the data may not be representative for patients who had mild to non-specific manifestations and therefore didn't require medical care in hospitals.

In conclusion, the presented results may help to develop an improved clinical score to differentiate between H1N1 influenza and dengue infection for early diagnosis and to guarantee an appropriate and swift treatment.

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Figures

Tables

Curriculum Vitae

PERSONAL INFORMATION

Name	Amrita Maria Wan-Lü-Tai Schäffler
Date of Birth	23.09.1986
Nationality	German
Civil status	Single
Current occupation	Student

EDUCATION

2004	graduated from BG/BRG Stainach
2007 – present	Medical University Graz, Study of Human Medicine

CERTIFICATES

2008	Certification of Common Invasive Procedures I and II
2008	First Diploma for the Study of Human Medicine (24.07.2008)
2012	Second Diploma for the Study of Human Medicine (29.06.2012)
2013	OGKA Diploma for Acupuncture

CLINICAL TRAINEESHIP

2009	LKH Rottenmann, Department of Surgery; 2 weeks
2010	UKH Graz, Hospital for Trauma Surgery; 6 weeks
2010	Nepean Hospital Penrith; Department of Neurology, Medical School of Sydney – Australia, 4 weeks
2012	LKH Graz, Department of Neonatology; 2 weeks
2012	Chulalongkorn Memorial Hospital, Department of Gynecology, Chulalongkorn University Bangkok – Thailand; 4 weeks

PRACTICAL YEAR

2012	RSUP Sanglah Hospital, Department of Pediatrics, Udayana University Denpasar - Bali/ Indonesia; 4 weeks
2012	General medicine, Dr. med. Adelheid Hopfer; 5 weeks
2013	Hospital zum heiligen Geist, Department of Internal Medicine, Frankfurt am Main – Germany; 6 weeks
2013	LKH Graz, University Hospital for Pediatric Surgery; 10 weeks

EMPLOYMENT HISTORY

2005	Office Management, Clinic of Dr. Hans Schäffler (fulltime)
2006-2011	Clinic of Dr. Hans Schäffler (part time, 8 hours/week)
2009-present	Hip Hop & House Dancing Classes, Sports Institute of Karl-Franzens-University Graz
2010-2013	Mercur Recreation, ego4you Kids (summer job)

SCIENCE WORK

2012-present	Diploma Thesis “Comparison of clinical and laboratory presentation between PCR confirmed H1N1 influenza infection, rapid diagnostic test confirmed dengue infection and blood smear confirmed plasmodium infection”

GRANTS

2010	Grant for International Rotations at the Nepean Hospital Penrith, Medical School of Sydney/ Australia by the Medical University of Graz
2012	Grant for International Rotations at the Chulalongkorn University Bangkok/ Thailand by the ASEA Uninet program
2012	Grant for International Rotations at the Udayana University Denpasar - Bali/ Indonesia by the Medical University of Graz

LANGUAGES

Mother Tongue	German
Foreign languages	English (fluent in spoken and written)
	French (basic)
	Italian (basic)

SPECIAL ABILITIES

2008	Basic Medical English I
2009	Basic Medical English II
2010	English in the Clinical Practice I

SKILLS/ INTERESTS

Computer Skills	Microsoft Office
Sports	Dancing (Street Dance Styles, Dance Instructor since 2009), Surfing, Snowboarding, Yoga
Traveling	