Original Article

Fever in infants under three months old: incidence of potentially serious bacterial infection and the usefulness of rapid diagnostic tests

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ABSTRACT

Aims: To assess the incidence and aetiology of potentially serious bacterial disease (PSBD), viral infection and viral-bacterial coinfection in a sample of febrile infants aged less than three months.

Methods: Prospective study of infants aged less than three months admitted to our hospital because of fever. A complete sepsis study was performed in all cases, with lumbar tap in selected cases. PSBD is defined as the growth of bacteria in blood, urine or cebrospinal fluid (CSF). The presence of Respiratory Syncitial Virus and of influenza A and B viruses was assessed during the epidemic period, and enteroviruses were investigated using the polymerase chain reaction in eleven patients aged less than one month. The SPSS 12.0 software was used for the analysis.

Results: The study population comprised 136 infants aged less than three months. Seventy patients (51.5%) met the Rochester low-risk criteria. The most common final diagnoses were non-focal febrile syndrome and urinary infection. PSBD was demonstrated in 33 cases (24.3%) (31 urine cultures, 2 CSF cultures and 7 blood cultures were positive). The incidence of documented viral infection was 30.8%. Viral and bacterial coinfection was demonstrated in 2 cases (4.8% of the total number of virus-infected patients). Enterovirus was demonstrated in the CSF in 5 (45.5%) of 11 cases studied; none of them had positive bacterial cultures.

Discussion: In the present study, urinary infection was the most frequently occurring potentially serious bacterial disease, with an incidence higher than those reported in other series. The inclusion of rapid virological diagnostic tests in the diagnostic algorithms helps in selecting those infants with lower risk of bacterial infection and opens new perspectives for the management of febrile infants aged less than three months.

Key Words: *Febrile infant. Bacterial infection. Rapid virological diagnostic tests.*

RESUMEN

Fiebre en el lactante menor de 3 meses: incidencia de enfermedad bacteriana potencialmente grave y utilidad de los test de diagnóstico rápido virológico

Objetivos: Determinar la incidencia y la etiología de la enfermedad bacteriana potencialmente grave (EBPG), de la infección vírica y de la coinfección (vírica y bacteriana) en una muestra de lactantes febriles menores de tres meses.

Métodos: Estudio prospectivo de lactantes menores de tres meses que ingresaron en nuestro centro por fiebre. A todos ellos se les realizó estudio completo de sepsis, con punción lumbar en casos seleccionados. Se define enfermedad bacteriana potencialmente grave como el crecimiento de un microorganismo bacteriano en sangre, orina o líquido cefalorraquídeo. Se determinó la presencia de virus respiratorio sincitial (VRS) y virus Influenzae A y B durante el período epidémico, y la presencia de enterovirus, por reacción en cadena de la polimerasa, en 11 pacientes menores de un mes. Para el análisis de los resultados se utilizó el programa SPSS (versión 12.0).

Resultados: Se incluyeron 136 lactantes febriles menores de tres meses. Cumplían los criterios de bajo riesgo de Rochester 70 pacientes (51,5%). Los diagnósticos finales más frecuentes fueron síndrome febril sin foco e infección urinaria. En 33 casos (24,3%) se demostró una EBPG (31 urocultivos, 2 cultivos de líquido cefalorraquídeo y 7 hemocultivos resultaron positivos). La incidencia de infección vírica demostrada fue del 30,8%. En 2 pacientes se demostró coinfección vírica y bacteriana (un 4,8% del total de los pacientes infectados por virus). Se detectó la presencia de enterovirus en líquido cefalorraquídeo en 5 (45,5%) de 11 casos estudiados, ninguno de ellos mostró cultivos bacterianos positivos.

Discusión: En nuestro estudio, la infección urinaria se muestra como la enfermedad bacteriana potencialmente grave con mayor incidencia, superior a la hallada en otras series. La incorporación a los algoritmos diagnósticos de los test de diagnóstico rápido virológico ayuda a seleccionar a los lactantes con menor riesgo de padecer enfermedad bacteriana, y abre nuevas perspectivas en el manejo del lactante febril menor de tres meses.

Palabras clave: *Lactante febril. Infección bacteriana. Test de diagnóstico rápido virológico.*

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INTRODUCTION

A high temperature is one of the main reasons people come to the paediatric emergency department. The cause of fever remains unclear in up to 20% cases after a complete medical history is taken and physical exam is carried out[1].

In infants under three months old the risk of potentially serious bacterial infection (PSBI) is higher than for other children and stands at 6-10% according to some series[2-4]. Infections affecting children of this age do not usually present many signs or symptoms and many are vague and vary widely. Therefore, different diagnostic and treatment markers have been investigated to help identify the patients who require a more aggressive initial approach[5]. Therefore, different scales have been designed, for example: the Rochester criteria (for infants ≤ 60 days old) which divides patients into high and low risk (Table 1); the Philadelphia protocol (infants between 29 and 60 days old); and the Boston criteria (infants between 28 and 89 days old)[6-9]. By using clinical criteria and tests it is possible to identify children who run a higher risk of having PSBI. Garra et al[9] confirmed the reliable negative predictive value of the Rochester and Philadelphia criteria when applied to different types of infant populations in a recent study (98.8% and 99.7% respectively).

New biochemical markers have recently been introduced (Interleukin-6, Procalcitonin, Tumoral Necrosis Factor) with the aim of identifying PSBI in the early stages[10,11]. Similarly, rapid diagnostic tests (RDT), (used mainly for RSV, influenza A and B viruses and enterovirus) are gradually being introduced into daily clinical practice and as a result many authors have highlighted the need to re-evaluate the existing protocol regarding the diagnosis and treatment of febrile infants[12,13].

The first aim of this study was to assess incidence rates in Spain and the causes of PSBI and viral infections in infants under three months old. The second aim was to identify co-infection incidence in cases in whom RDT were carried out in order to establish whether confirming a viral cause meant a reduction in the risk of PSBI.

TABLE 1. Rochester criteria for bacterial infection

1. General good state
2. Previously healthy:
   - Born at term ≥ 37 weeks gestation
   - No perinatal antibiotic therapy
   - No treatment for unexplained hyperbilirubinemia
   - No previous hospitalisation
   - No chronic diseases or birth conditions
   - Not hospitalised for longer than the mother
3. No evidence of infections of the skin, soft tissues, bones or ears.
4. Lab values:
   - Leukocytes 5000- 15000/mm^3
   - Absolute band count < 1500/mm^3
   - ≤ 10 leukocytes per high power field in urine sediment
   - ≤ 5 leukocytes per high power field in faeces

PATIENTS AND METHOD

A prospective study was carried out on 136 infants under three months old admitted to Short Stay and Hospitalisation Units, from our emergency department between 1st September and 31st December 2003 with an axillary temperature of 38°C or above. Tests had been also been carried out in all subjects. Despite the fact that rectal temperature is the most reliable indicator for children of this age, the axillary temperature taken using a digital thermometer was used for this study because it was the easierst and most comfortable option for the patient. Infants with chronic diseases were excluded from the study.

PSBI was defined as the growth of bacteria in a biological liquid that is normally sterile [cerebrospinal fluid (CSF), blood or urine]. The Rochester criteria was used to assess whether the risk of PSBI was high or low in infants aged up to three months old.

Our normal procedure when dealing with febrile infants under three months old includes carrying out additional tests and admitting the patient so that they can be observed and/or treated if necessary according to current hospital guidelines (Figure 1)[14]. A complete sepsis study was carried out in patients under one month old as were the following tests: a complete blood test, C-reactive protein (CRP), qualitative procalcitonin (PCTq), ionogram and acid-base balance (ABB); blood cultures; urine sediment obtained using a catheter and urine cultures; lumber tap with biochemical analysis, Gram staining and CSF culture. All the same tests, apart from the lumber tap, were carried out in infants aged between one and three months. The lumber tap was only performed in selected cases and depended on the clinical test results, risk factors and the urine sample collected in the first instance. A second sample was collected using a catheter when abnormal results were obtained.

RDT were requested for 64 patients according to the criteria of the doctor in charge of the patient at that time in order to determine the origin of the fever and/or assess more or less

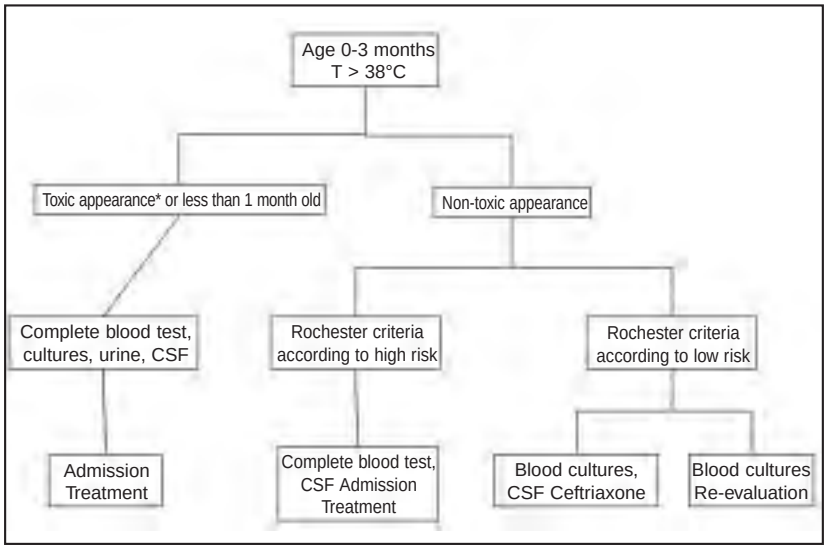

Figure 1. The diagnostic approach in a child with fever up to the age of three months. *Toxic appearance: a patient whose condition is affected generally or has sepsis. CSF: Cerebrospinal fluid.

evident respiratory symptoms. The presence of RSV (RSV-Direct IF identification; Bio-Mériaux®) and influenza A and B viruses (Directigen™ Flu A + B: BD) was identified in nasopharyngeal aspiration during the epidemic period.

Enterovirus was retrospectively investigated using polymerase chain reaction (PCR-EV) in order to obtain a clearer idea as to the cause of the fever in patients under one month old who had provided a CSF sample and had been discharged with a diagnosis of non-focal febrile syndrome. The PCR technique was carried out with the Pan-Enterovirus reading system using the Laboratory Light-Diagnostics kit.

The results were analysed using SPSS software (version 12.0).

RESULTS

A total of 136 febrile infants were included in this study with ages ranging from 4 days to 3 months (57.4% were male); 61 (44.8%) were under one month old. The average age was 39.6 ± 23.3 days old. Seventy patients (51.5%) fulfilled the Rochester criteria for bacterial infection. The final most common diagnoses are detailed in Table 2. None of the patients included in the study had received treatment with antibiotics previously.

PSBI was detected in 33 patients: positive results were obtained from urine cultures in 31 cases (29 *Escherichia coli*, 1 *Klebsiella pneumoniae* and 1 *E. coli* and *Proteus mirabilis* co-infection); positive results were obtained from 2 CSF cultures and 7 blood cultures. Of the 7 positive blood cultures, 5 revealed urinary tract infection (UTI) caused by *E. coli* with bacteraemia, 1 patient had UTI caused by *Klebsiella pneumoniae* and secondary bacteraemia and the seventh blood culture revealed sepsis caused by *S. agalactiae.* Of the two positive CSF cultures, one showed sepsis caused by *S. agalactiae* and the other, meningitis caused by group B *Neisseria meningitidis.*

Of the total number of patients who underwent RDT (64 patients), viral infection was confirmed in 65.6% of cases and therefore the incidence for the whole sample was 30.8%. Infection caused by RSV was confirmed in 26 patients, while infection by the influenza virus was the result in 11 patients, and 5 patients had infection by enterovirus. Eleven patients were able to provide a substantial CSF sample and were im-

TABLE 2. Final diagnoses

Diagnosis	Incidence	%
Febrile syndrome	37	27.2
Urinary infection	31	22.8
Infection caused by RSV	26	19.1
Infection caused by influenza virus	11	8.1
Upper respiratory tract infection	8	5.9
Otitis media	5	3.7
Infection caused by enterovirus	5	3.7
Acute gastroenteritis	3	2.2
Negative RSV bronchitis	3	2.2
Bacterial meningitis	2	1.5
Lymphocytic meningitis	1	0.7
Pneumonia	1	0.7
Other diagnoses	3	2.2
Total	136	100

RSV: Respiratory Syncitial Virus.

mediately diagnosed with non-focal febrile syndrome. RDT were carried out retrospectively to detect enterovirus and positive results were obtained in 5 cases (45.5%). The biochemistry was normal for these patients and none had pleocytosis. All received antibiotic treatment (ampicillin and gentamicin, cefotaxime or ceftriaxone) while they were waiting for the culture results. No positive cultures were obtained from any of the 11 patients who provided a substantial CSF sample. All the epidemiological information, details about the treatment received, patient management and results relating to this sample can be found in Tables 3 and 4.

Two patients demonstrated viral-bacterial co-infection (4.8% of those infected by viruses). In both cases the PSBI was a UTI and the viruses detected were RSV in one infant with a bronchiolitis and influenza A virus in another patient. Both patients evolved well and the worst prognosis was isolated PSBI.

DISCUSSION

The incidence of PSBI in our series was 24.3% of the whole sample. This result is higher than that of other clinical reviews (12.6% for new borns aged between 3 and 2 days[15]; 10.7% for infants aged between 29 and 60 days[16]) and similar to results published in other recent studies[9,17], in which authors identified an incidence of PSBI of around 25%. In our study UTI was the most common PSBI among febrile infants (22.8%), which coincides with the findings of other published series. However, we should not overlook the fact that viral infection is still the most common cause of fever in this age group and is the most commonly attributed respiratory virus. Most of these viruses are not usually studied in medical practice (adenovirus, coronavirus, rhinovirus) although in the last few years RDT have been used more regularly, for example RSV and influenza A and B virus detection in respiratory secretions during an epidemic period and enterovirus detection by analysing different fluid samples.

Our sample presented an incidence of viral infection of 30.8% and this figure may be even higher given that RDT were not carried out in all the patients included in the study. Hsiao, et al obtained similar results using the same techniques. Respiratory viruses were the cause of fever in a third of infants under 6 months old (38%)[18]. These tests vary a great deal in sensitivity, accuracy and in how quickly the results can be obtained[19] depending on the type of technique used (direct or indirect immunofluorescence staining, polymerase chain reaction in real time (PCR-RT), enzyme immuno-assay (EIA) and rapid antigen detection). These techniques are very useful tools for managing the diagnosis and treatment of this group of patients in the paediatric emergency department. Their are use, decreases antibiotic treatment and increases the use of specific antiviral drugs. The number of unnecessary additional tests carried out also decreases as does the number of patients admitted, days hospitalised and consequently, the direct and indirect costs that this type of patient generates[20]. The fact that only 2 of our patients had bacterial and viral infections (4.8% of with viral infections) compared to almost 25% of the whole sample that had a PSBI, coincides with the findings of previous studies (ranging between 4.2% and 7%)[21-23]. In these studies the authors concluded that the prevalence of PSBI is low among febrile infants with proven viral infections. Thus, the presence of a viral infection would identify the febrile infants

TABLE 3. Epidemiological data treatment and management and PCR-EV results of CSF in 11 patients

Patient	Age	Sex	EV	ABT	Days ABT	Days admitted
1	6	M	+	A+G	4	6
2	8	M	-	Ceft	2	4
3	11	M	+	Ceft	1	1
4	13	M	-	Cefo	3	4
5	17	M	-	A+G	2	4
6	18	F	-	Ceft	3	5
7	22	M	+	Ceft	1	2
8	24	F	+	Ceft	1	4
9	26	M	-	Ceft	2	3
10	28	M	+	A+G	4	9
11	31	M	-	Ceft	1	2

Age: days; Sex: M = male, F = female; EV: enterovirus detected in the cerebrospinal liquid; ABT: antibiotic treatment (A+G = ampicillin+gentamicin, ceft = ceftriaxone; cefo = cefotaxime).

TABLE 4. Blood analysis and microbiological test results of the 11 patients undergoing PCR-EV in CSF

Patient	Leukocytes (cells/mm³)	Band count (cells/mm³)	CRP	PCTq	Cultures	CSF leukocytes
1	9300	0	12	–	Negative	0
2	9100	728	13.5	–	Negative	5
3	8400	420	18.1	< 0.5	Negative	10
4	9200	736	9.7	–	Negative	10
5	10600	0	27	0.5-2	Negative	10
6	12200	0	13.5	–	Negative	10
7	9400	470	8.8	–	Negative	5
8	11500	0	2.5	< 0.5	Negative	25
9	12000	720	9.1	< 0.5	Negative	0
10	4400	440	19.2	–	Negative	0
11	9400	470	8.8	–	Negative	5

CRP: C-reactive protein (normal values up to 15mg/l); PCTq: qualitative procalcitonin (values suggestive of PSBI > 2 ng/ml); CSF: cerebrospinal fluid; cultures: blood culture, urine culture, CSF culture.

with a lower risk of having PSBI, given that the infants with unconfirmed viral infections were more likely to have PSBI[22,23]. The same occurs when positive results are obtained for influenza A and B viruses in which the prevalence of bacteraemia, urinary infections, pneumonia and PSBI is lower[24]. However, most of authors have noted that the rate of UTI is not insignificant in infants with viral infections (5.4%) and therefore it is recommended that all patients should at least have their urine tested[21].

Apart from RSV detection, the presence of other viruses such as influenza and EV has also been studied in this study. Infants with infection caused by EV may not show any symptoms and it may develop in the same way as a benign illness or severe sepsis. Detection using new less aggressive diagnostic methods could change the approach to diagnosis and treatment of these infants and could shorten their stay in hospital[25]. The presence of viral infection by EV in the CSF of 11 infants under the age of one month with normal cellularity and biochemistry is of note. It would not have been possible to know whether the fever was caused by a viral infection or not without using RDT. Byington et al[26] carried out a study in order to assess the epidemiological prevalence of infection caused by EV in the central nervous system using a sample of 345 (febrile and afebrile) infants under 90 days old. EV was detected in 25.8% of cases, whereas only 6.6% demonstrated concomitant PSBI. In our sample of 11 patients, infection by EV in the CSF was demonstrated in 45.5% of cases and none had a PSBI. In line with recently published studies[27,28], these authors have also demonstrated that the use of RDT along with the Rochester criteria helped us to identify infants with a lower risk of invasive illness[23].

Despite the fact that our results are similar to what has already been published, we are aware that the size of our sample makes it is difficult to draw conclusions. Given that there were only two cases of bacterial and viral co-infection we cannot assess the impact of each of these viruses separately. We would also need to consider the fact that the incidence of these infections could vary if the study were extended to cover a whole year, given the seasonal distribution of certain infections. RSV and the influenza virus have not been identified in all patients included in this study. The following viruses that also commonly affect children were also excluded: herpesvirus 6, rotavirus, rhinovirus, coronavirus and other viruses that have recently been discovered such as human metapneumovirus and human bocavirus. These last viruses were not included because most cannot be diagnosed quickly and the ability to diagnose them is currently not considered of utility in daily clinical practice. Thus, the incidence of detected viral infection could be much higher if all the RDT were carried out. It would be interesting to design new studies to assess the incidence of these viral infections and their impact on PSBI incidence rates in febrile infants.

To summarise, on analysing the epidemiological results obtained, we believe that the use of new diagnostic techniques such as RDT will allow a less aggressive approach in the management of three-month-old febrile infants to be adopted in the near future (fewer invasion tests, fewer days in hospital and less treatment with antibiotics). This will subsequently reduce medical costs without increasing the risk for the patient. Finally, we would like to propose a possible diagnostic and treatment algorithm for non-focal fever in infants under three months old which has already been implemented in our department as part of the standard protocol.

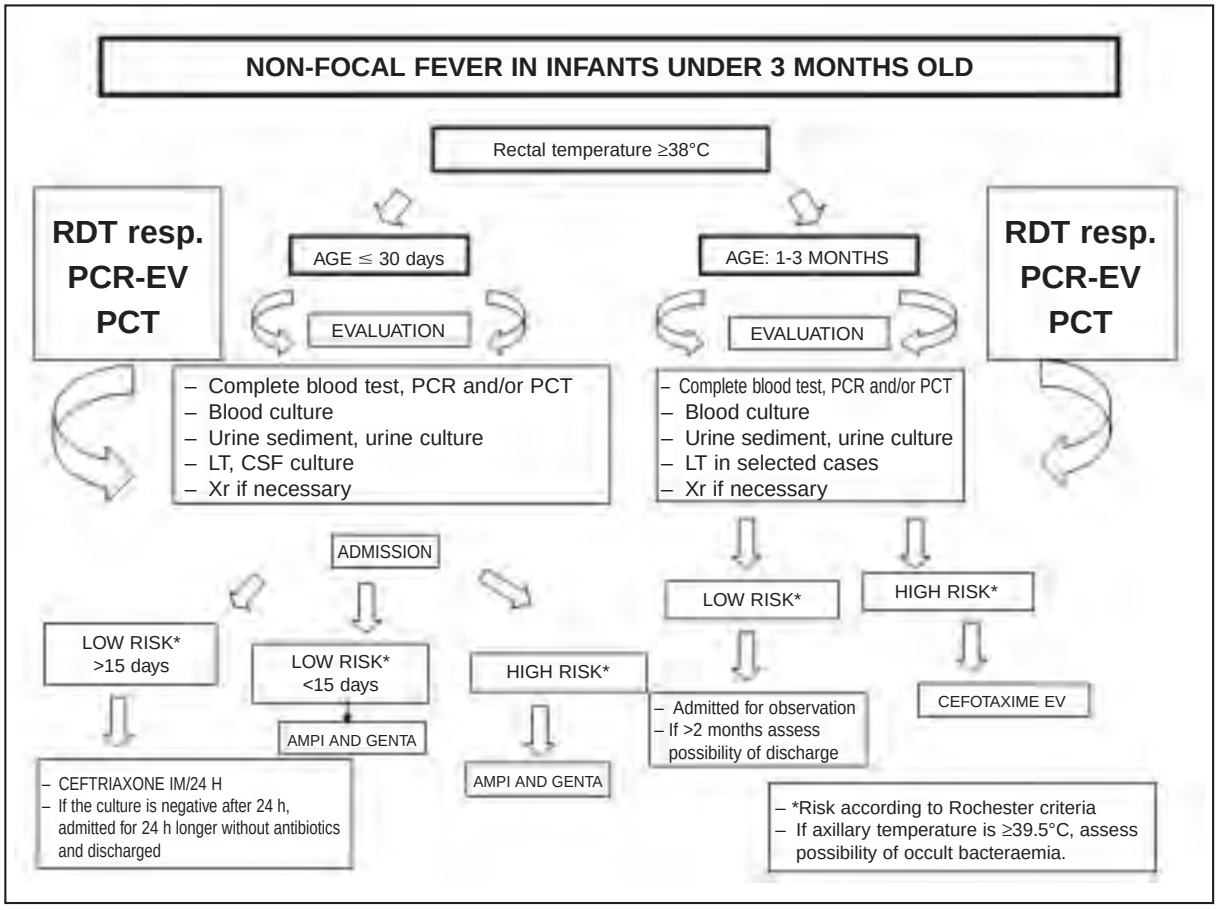

Figure 2. Proposed diagnostic and treatment protocol of non-focal fever affecting infants under 3 months old.

RDT resp: respiratory virus rapid diagnostic tests; PCR-EV: polymerase chain reaction for enterovirus; PCT: procalcitonin; PCR: reactive C protein, PL: lumber tap; CSF: cerebrospinal fluid; Xr: X-ray; AMPI + GENTA: ampicillin plus gentamicine.

REFERENCES

1- Lee GM, Harper MB. Risk of bacteriemia for febrile young children in the post-*Haemophilus influenzae* type b era. Arch Pediatr Adolesc Med 1998;152:624-8.

2- Baraff LJ. Management of fever without source in infants and children. Ann Emerg Med 2000;36:602-14.

3- Bachur R, Harper M. Predictive model for serious bacterial infections among infants younger than 3 months of age. Pediatrics 2001;108:311-6.

4- Slater M, Krug SE. Evaluation of the infant with fever without source an evidence based approach. Emerg Med Clin North Am 1999;17:97-126.

5- Pantell RH, Newman TB, Bernzweig J, Bergman DA, Takayama JI, Segal M, et al. Management and outcomes of care of fever in early infancy. Jama 2004;291:1203-12.

6- Bonadio WA. Keeping febrile young infants out of the hospital. Contemporary Pediatrics 1994;11:73-8.

7- Kadish HA, Loveridge B, Tobey J, Bolte RG, Corneli HM. Applying outpatient protocols in febrile infants 1-28 days of age: can the threshold be lowered? Clinical Pediatrics (Phila) 2000;39:81-8.

8- Jaskiewicz JA, McCarthy CA, Richardson AC, White KC, Fisher DJ, Dagan R, et al. Febrile infants at low risk for seriours bacterial infection-an appraisal of Rochester criteria and implications for management. Pediatrics 1994;94:390-96.

9- Garra G, Cunningham SJ, Crain EF. Reappraisal of Criteria Used to Predict Serious Bacterial Illness in Febrile Infants Less than 8 Weeks of Age. Acad Emerg Med. 2005;12:921-5.

10- Fernández A, Luaces C, Garcia JJ, Fernandez J. Procalcitonin in pediatric emergency departments for the early diagnosis of invasive bacterial infections in febrile infants: results of a multicenter study and utility of a rapid qualitative test for this marker. Pediatr Infect Dis J 2003;22:895-903.

11- Malik A, Hui CP, Pennie RA, Kirpalani H. Beyond the complete blood cell count and C-Reactive protein. A systematic review of modern diagnostic tests for neonatal sepsis. Arch Pediatr Adolesc Med 2003;157:511-6.

12- Dorfman D, Bauchner H. The impact of new diagnostic test on the management of children with fever. Arch Pediatr Adolesc Med 2000; 154:761-2.

13- Diomandé D, Bellavoine V, Gilles I, Gehanno B, Checoury A, Pascal C. Bénéfice de l'utilisation saisonnière d'un Test de diagnostic rapide (TDR) de la grippe aux urgences pédiatriques. Arch de pédiatr 2006;13:1463-5.

14- Pou J. Urgencias en Pediatría. Protocolos diagnóstico-terapéuticos. Unidad Integrada Hospital Clínic-Sant Joan de Déu. 4ª Edición. Ergon. Barcelona 2005.

15- Baker MD, Bell LM. Unpredictability of serious bacterial illness in febrile infants from birth to 1 month of age. Arch Pediatr Adolesc Med 1999;153:508-11.

16- Baker MD, Bell LM. The management of 1-2 month old febrile infants: an 8-year report care. Paper presented at: 38th Annual meeting of the ambulatory Pediatric Associaton. May 1998;New Orleans, LA.

17- Gajdos V, Foix L, Mollet-Boudjemline A, Perreaux F, Trioche P, Labrune P. Facteurs prédictifs de la présence d'une infection bactérienne sévère chez les nourrissons fébriles âgés de mois: analyse multivariée. Arch de Pediatrie 2005;12:397-403.

18- Hsiao A, Chen L, Baker D. Incidence and predictors of serious bacterial infections among 57-to 180-day-old infants. Pediatrics 2006;5:1695-701.

19- Vega R. Rapid viral testing in the evaluation of the febrile infant and child. Curr Opin Pediatr 2005;17:363-7.

20- Henrickson K. Cost-effective use of rapid diagnostic techniques in the treatment and prevention of viral respiratory infections. Pediatr Annals 2005;34:24-31.

21- Purcell K, Fergie J. Concurrent serious bacterial infections in 2396 infants and children hospitalized with VRS lower respiratory tract infections. Arch Pediatr Adolesc Med 2002;156:322-32.

22- Levine DA, Platt SL, Dayan PS, Macias CG, Zorc JJ, Krief W et al. Risk of serious bacterial infection in young febrile infants with respiratory syncytial virus infections. Pediatrics 2004;113:1728-34.

23- Byington CL, Enriquez FR, Hoff C, Tuohy R, Taggart EW, Hillyard DR et al. Serious bacterial infections in febrile infants 1 to 90 days old with and without viral infections. Pediatrics 2004;113:1662-6.

24- Smitherman H, Caviness AC, Macias CG. Retrospective review of serious bacterial infections in infants who are 0 to 36 months of age and have Influenza A infection. Pediatrics 2005;115:710-8.

25- Abzug MJ. Presentation, diagnosis, and management of enterovirus infections in neonates. Paediatr Drugs 2004;6:1-10.

26- Byington CL, Taggart EW, Carrol KC, Hillyard DR. A polymerase chain reaction-based epidemiologic investigations of the incidence of non-polio enteroviral infections in febrile and aferile infants 90 days and younger. Pediatrics 1999;103:E27.

27- Kawashima H, Kobayashi K, Aritaki K, Takami T, Ioi H, Kashiwagi Y, et al. Diagnosis and evaluation of febrile infants under 4 months of age in Japan by using RT-PCR for enterovirus. Journal of Infection 2006;53:16-20.

28- Rittichier KR, Bryan PA, Bassett KE, Taggart EW, Enriquez FR, Hillyard DR, et al. Diagnosis and outcomes of enterovirus infections in young infants. Pediatr Infect Dis J 2005;24:546-50.