Rapid Adenovirus typing method for species identification

Fabienne Rayne[a], Linda Wittkop[b,c], Clément Bader[c], Somar Kassab[a], Camille Tumiotto[a,e], Sylvie Berciaud[d], Harald Wodrich[a], Marie-Edith Lafon[a,e,*], the Typadeno Study Members[1]

[a] Univ Bordeaux, CNRS, UMR 5234 Fundamental Microbiology and Pathogenicity, F-33000, Bordeaux, France
[b] Univ Bordeaux, ISPED Inserm, Bordeaux Population Health Research Center, team MORPH3EUS, UMR 1219, CIC-EC 1401, F-33000 Bordeaux, France
[c] CHU de Bordeaux, Pôle de santé publique, Service d'information médicale, F-33000 Bordeaux, France
[d] CHU de Bordeaux, Pôle de Pédiatrie, F-33000 Bordeaux, France
[e] CHU de Bordeaux, Pôle de Biologie et Pathologie, Laboratoire de Virologie, F-33000 Bordeaux, France

A B S T R A C T

Adenoviruses are characterized by a large variability, reflected by their classification in species A to G. Certain species, eg A and C, could be associated with increased clinical severity, both in immunocompetent and im- munocompromised hosts suggesting that in some instances species identification provides clinically relevant information. Here we designed a novel "pVI rapid typing method" to obtain quick, simple and cost effective species assignment for Adenoviruses, thanks to combined fusion temperature (Tm) and amplicon size analysis. Rapid typing results were compared to Sanger sequencing in the hexon gene for 140 Adenovirus-positive clinical samples included in the Typadeno study. Species A and C could be identified with a 100% positive predictive value, thus confirming the value of this simple typing method.

Adenoviruses have been known for decades as respiratory and digestive pathogens, common in infants and children (Echavarria, 2008; Walls et al., 2003; Wang et al., 2010). However, adenoviral opportunistic infections in immunocompromised hosts now represent an emerging medical problem, since primary infections and viral reactivations induce significant morbidity and mortality in patients such as stem cell recipients. In this setting, 4–9% of adults and 20–26% of children experience Adenovirus-induced disease, mostly expressed as interstitial pneumonitis, hemorrhagic colitis, or disseminated multiorgan infection. Death rates may reach 8–26% of the patients. Adenoviral viremia occurs most frequently during the first term following transplantation (median: 49th day) (Lion et al., 2003; Kalpoe et al., 2007).

Diagnostic procedures improved concomitantly: quantitative real time PCR assays are now widely available. They may be applied to blood and several other body fluids or biopsies (Echavarria, 2008; Lion et al., 2003; Kalpoe et al., 2007; Lindemans et al., 2010; Baldwin et al., 2000; Leruez-Ville et al., 2004). Early adenoviral detection may be attained by DNA detection in stools, where viral shedding is always precocious and prolonged. PCR may help in predicting a poor clinical outcome both when adenoviral DNA load exceeds 10^6 copies/gram in stools (increased risk of viremia) (Lion et al., 2010), 10^6 copies/mL in whole blood (increased risk for fatal complications) (Heim, 2011) or display rapidly ascending kinetics in blood (Heim, 2011).

Adenoviruses are characterized by an important genomic and antigenic variability, leading to the present description of 69 types dispatched within 7 species. Simultaneous infection with different adenoviral species is observed in 30% of the clinical reports and various species may induce successive episodes in 36% of patients (Echavarria, 2008). Various recent reports have described the molecular epidemiology of Adenovirus infections around the world, especially in children with respiratory infections (Qurei et al., 2012; Yun et al., 2015; Cheng et al., 2015; Yamamoto et al., 2014; Demian et al., 2014; Abbas et al., 2013; Sriwanna et al., 2013; Wang et al., 2015; Lai et al., 2013; Li et al., 2015; Berciaud et al., 2012). However, species A and C seem the most prevalent, although estimates vary according to the population (immunosuppressed or immunocompetent patients, children or adults): for species A they range from 3.4% to 23% (Lion et al., 2010), and for species C from 38% (Baldwin et al., 2000) to 77% (Lion et al., 2010). In our own hospital setting, species C was estimated to cause 63% of adenoviral infections over three consecutive years (Berciaud et al., 2012).

Severe respiratory infections with species B serotype 7 or 3 Adenoviruses have been described in several instances (Yamamoto

Table 1
Primers used for Adenovirus rapid typing method.

Primer name	Primer Sequence
5′-pVI-ABEFG	5′ GAGCCGCCCTCTTATGAGGAGGCATTGAAACACGG 3′
5′-pVI-.CD	5′ GAGCCTCCCTCGTACGAGGAGGCCCTAAAGCCCGG 3′
3′-pVI-generic	5′ CCCAGACCCACGATGCTGTTCAGAGTGCTTTGCCAGT 3′

et al., 2014; Lai et al., 2013; Gray et al., 2007). However, all species may be menacing to immunocompromised hosts or patients affected with an underlying disease. Indeed, species C and species A have been associated with higher risk of viremia and clinical severity (Leruez-Ville et al., 2004; Lion et al., 2010; Gray et al., 2007; Kroes et al., 2007; Leruez-Ville et al., 2006), especially in immunosuppressed patients (Madisch et al., 2006), earlier onset of infection after transplantation (Kroes et al., 2007; Leruez-Ville et al., 2006) and severe chronic lung disease (Wurzel et al., 2014). Thus, specific "genotype" assessment is currently dispensable, whereas Adenovirus species determination could bring relevant information.

Therefore, patients monitoring based on combined viral load measurement by quantitative PCR and species determination may be suggested, although species determination is not yet commonly available in diagnostic virology laboratories.

In most available studies, Adenovirus typing is based upon sequencing (Madisch et al., 2006), a method which may not be routinely available in diagnostic settings or is considered too costly. We therefore aimed at designing a new "rapid typing method", which was tested on all samples belonging to a single patient, then to 140 samples included in the monocentric observational cohort diagnostic study named Typadeno, conducted within the Bordeaux University Hospital and authorized by the regional ethical committee.

The rapid typing method relies on the association of real-time SybrGreen PCR with fusion temperature (Tm) analysis and agarose gel electrophoresis. Two sets of primers were designed to amplify a region in the gene encoding the capsid protein VI (pVI gene), which displays significant variability, in terms of sequence and size between different viral species but not within genotypes of a single species. These sets of primers differed only by the forward primer (Table 1). The first couple was designed to amplify preferentially ABEFG species (5′-pVI-ABEFG primer) and the second CD species (5′-pVI-CD). Each forward primer presented a maximum of 4 or 8 mismatches with standard ABEFG or CD species sequences, respectively (Table 2). The reverse primer displayed a maximum of 4 mismatches depending on Adenovirus species. Amplification conditions were optimized in order to allow PCR reaction in the same apparatus with both sets of primers to facilitate further analysis. The PCR reaction was performed in 25 µl containing 0.2 µM of each primer, 5 µl of matrix DNA and 12.5 µl of Maxima™SybrGreen/Rox qPCR Master Mix 2X (Fermentas). The amplification was conducted in MyIQ real time PCR thermocycler (Biorad) as followed: activation at 95 °C for 3 min and 42 amplification cycles at 95 °C for 10 s (s), 50 °C for 30 s and 72 °C for 30 s (measure fluorescence). The melting curve was determined by denaturing amplification products at 95 °C for 30 s, annealing at 50 °C for 1 min and increasing 0.5 °C for 10 s 100 times (fluorescence measurement). The amplicon size was then determined on 2.5% agarose gels. The pVI rapid typing method was first validated by our group on reference Adenoviral strains (plasmids with pVI gene from different species), thus enabling the design of precise recommendations for Tm and fragment sizes, according to Adenovirus species (Fig. 1 and Table 2). Combined analysis of Tm and fragment size for each sample allows an easy determination of Adenovirus species, A, B and C. Due to close characteristics, possible confusions may arise for Adenoviruses 40 (species F) and 3 (species B). Fig. 1 shows examples for four different patient samples, with B and C Adenovirus species identification.

Table 2
Adenovirus species identification parameters using pVI rapid typing method. For each species and serotype, the expected PCR product size (in base pairs) and Tm are indicated. Tm was obtained with the species corresponding 5′ primers. The inner columns specify the number of primer mismatches for various Adenovirus serotypes. For Ad40 and Ad52, Tm could not be determined because no cloned pVI gene was available.

Species	Serotype	Primer mismatches			Size (bp)	Tm (°C)
		5′-pVI-ABEFG	5′-pVI-CD	3′-pVI-generic		
A	Ad12	7	11	7	311	85.5–86.5
	Ad18	6	10	4	296	85.5–86.5
B	Ad3	7	7	1	278	89–89.5
	Ad11	8	12	3	266	88
	Ad14	8	12	3	266	88
C	Ad1	8	3	1	281	91.5
	Ad5	7	3	1	281	91–92
D	Ad9	8	4	6	245	93
	Ad15	7	4	5	242	92.5
	Ad19a	8	3	5	242	92
	Ad19p	7	4	6	242	91.5–92
	Ad37	8	4	5	242	91.5
E	Ad4	8	9	1	248	91.5
F	Ad40	7	8	3	278	
	Ad41	7	8	5	285	88.6
G	Ad52	8	7	3	314	

Fig. 1 shows examples for four different patient samples, with B and C Adenovirus species identification.

In a first step, this method was applied to different biological fluids belonging to a single patient, sampled at different time points: typing was successful both in blood, stools, urine, liver biopsy (Fig. 2). Interestingly, the same Adenovirus species was detected, namely species C. In order to compare the rapid typing method to a reference technique, samples were submitted to Sanger nucleotide sequencing in the hexon hypervariable region 7 (Sarantis et al., 2004), followed by automatic electrophoresis on a 3500xL Genetic Analyser (Applied Biosystems) and related SeqScape v2.7 software sequence analysis. Hexon sequencing confirmed the rapid typing as Adenovirus type 5.

Finally, clinical samples collected between 2008 and 2012 were tested in Typadeno study. Samples diagnosed as Adenovirus-positive were included, when viral load was superior or equal to 10^4 copies/mL, copies/gram (stools) or copies/µg DNA (biopsies). Exclusion criteria comprised insufficient sample volume and inadequate freezing conditions. Sample size was estimated to be 148 samples in order to obtain a 10% precision for the estimation of the rapid test sensitivity of 95% for species C and under the hypothesis that the prevalence of species C is 38% (Epi Info 6 (v6.04fr) software). We calculated sensitivity, specificity, positive and negative predictive values and the 95% confidence intervals.

Sample selection was based upon a quantitative real time PCR assay targeting the hexon gene (Heim et al., 2003) (Roche: Magnapure 32 automatic extraction with Total nucleic acid isolation kit, Fast Start DNA master hybridization probes amplification kit on LightCycler 2.0) with an external standard curve (plasmid encoding hexon provided by H Wodrich). This method, which allowed linear quantification between 500 and 10^8 copies in our setting, has been repeatedly validated by the laboratory's participation to the annual european External Evaluation of Quality program "Quality Control for Molecular Diagnostics". Selected samples were processed at the Bordeaux University Hospital Virology Laboratory and at CNRS UMR 5234 Unit (Bordeaux University). Patients characteristics were collected based on a standardized record form.

A total of 140 samples were included, among which 53.6% stools, 29.3% respiratory and 5.7% whole blood samples. Careful retrospective collection of clinical, biological and serological data was obtained, with special emphasis on the immunological status of the patients and the existence of underlying chronic pathology. Altogether, a single sample was available in 92.8% of the patients, who were dominantly children (mean age 11.9 years, SD19.5), with a rather balanced sex ratio (53.6%

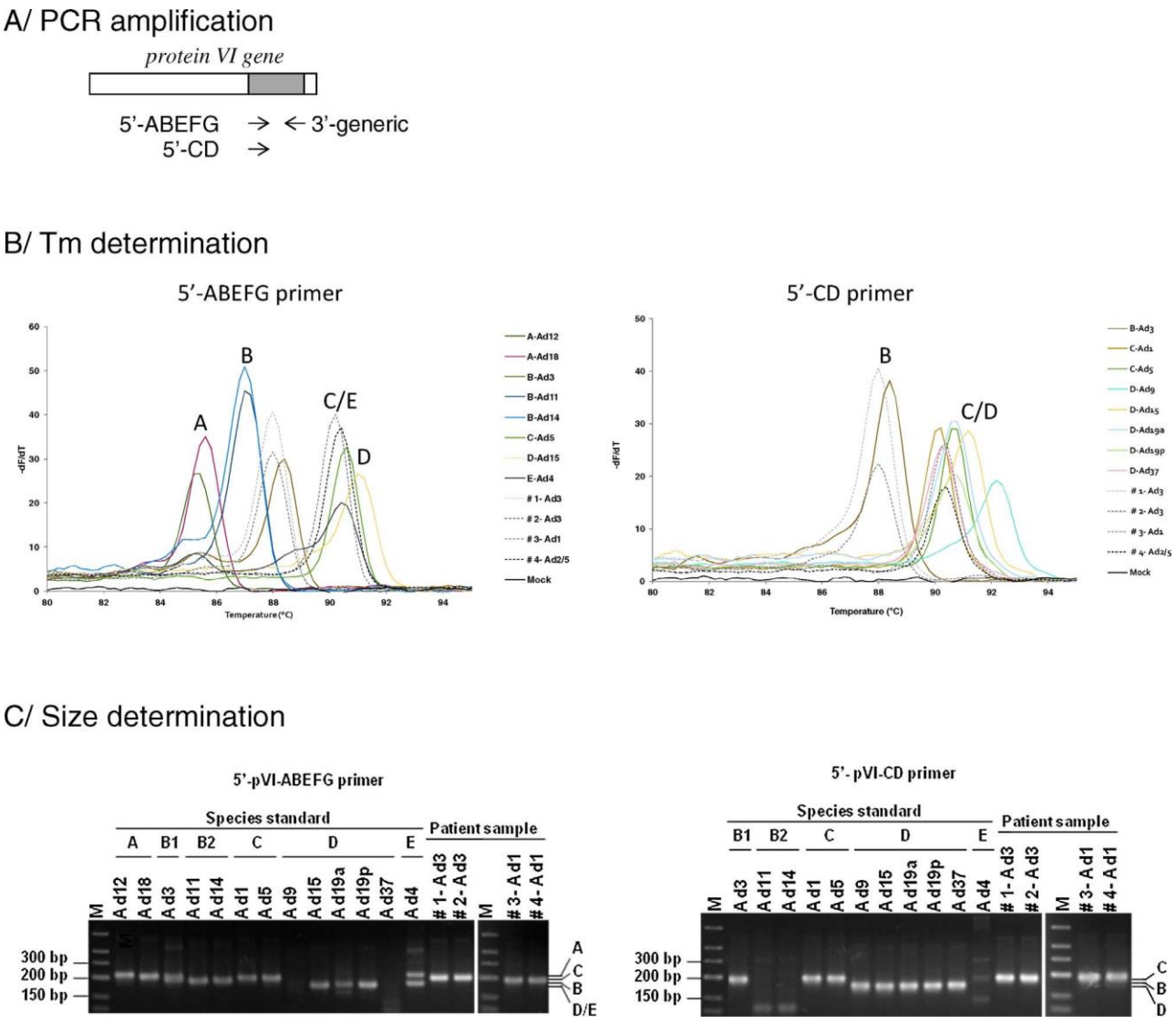


Fig. 1. A location of the amplified fragment on pVI gene. B: Tm determination with the two 5′primers according to AdV species C: Fragment size determination by agarose gel electrophoresis.

male). Although most could be considered immunocompetent (74.4%), an underlying disease was frequently noted (64%), such as various haemopathies, asthma (4.6%), eczema (2.3%) or gastro-oesophagal reflux (2.3%). Severity criteria were defined in immunocompetent patients (unplanned stay in hospital over 24 h, intra-venous therapeutic infusion) and in the immunocompromised (Cidofovir prescription, respiratory assistance, entero-invasive syndrome, Graft Versus Host Disease). They were frequently encountered (82.8% and 56.3% respectively in immunocompetent and immunocompromised patients), as well as bacterial or parasitic co-infections (61.5%). Most bacteria belonged to commensal flora, but Campylobacter jejuni, Haemophilus influenzae, Moraxella catarrhalis and Staphyloccocus aureus were occasionally detected. Candida albicans was also encountered (4.1%)

The pVI rapid typing method achieved interpretable results in 104 samples, which could be unequivocally confirmed by Sanger hexon sequencing in 43 cases, while remaining sequence reactions failed.

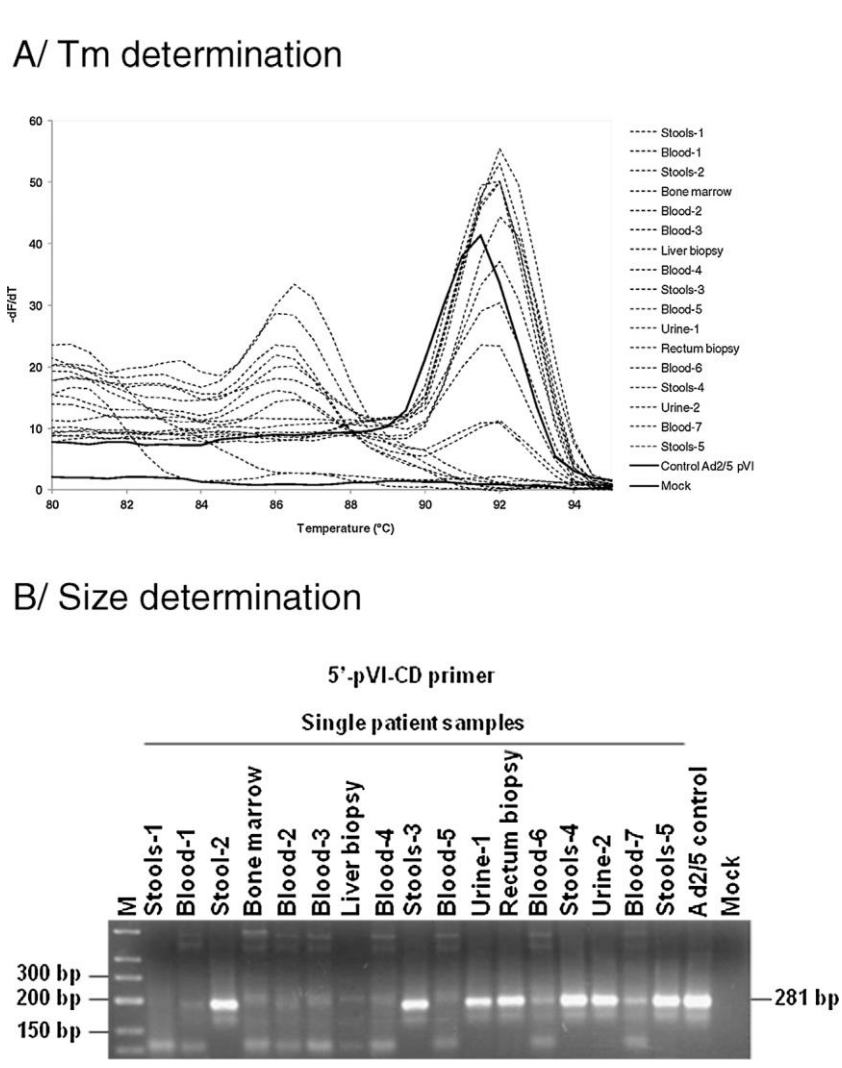
In total, both tests (the gold standard test – Sanger method and the pVI rapid test) were available in 55 patients. Prevalences of serotypes identified with the Sanger method in this subset of patients are presented in Table 3. Serotypes C, B and F were the most frequent ones with prevalences of 38%, 22% and 22%, respectively.

PVI typing proved particularly efficient for species C, with sensitivity, specificity, with positive predictive values and negative predictive values of 100% [95%CI: 74–100], 100% [95%CI: 90–100] 100% [95%CI: 84–100] and 100% [90–100], respectively (Table 3). pVI was also performant for other serotypes as shown in Table 3 but some estimations have large confidence intervals due to the low frequency of some of the serotypes. The interpretation of the rapid typing method for the 32 non-sequenced samples identified as A or C was clear cut.

Finally, neither Sanger sequencing nor the rapid typing method did identify any multiple infection (Gray et al., 2007).

Typadeno study presented certain limitations. The number of clinical samples included and tested was insufficient for D, E, F and G Adenovirus species, thus impairing the obtention of conclusive data. Furthermore, the restricted sample size for the subset of patients with both tests available led to large confidence intervals and thus low precision of our estimates, especially for serotypes with low abundance. Likewise, the threshold viral load necessary for an efficient typing (10^4 copies/mL) may appear limiting. However, our previous study indicated that most patients treated in our university hospital setting were infected with species A, B and C Adenoviruses (Berciaud et al., 2012) and elevated viral load, which is an important prognostic marker (Heim, 2011), is frequently noted in the severely infected patients for

Fig. 2. Rapid typing method applied to different body fluids from a single patient, with Tm (A) and size (B) determination.



whom our method may prove useful. Finally, the sequencing reference method chosen for our study, performed with the locally available reagents, unfortunately did not allow thorough typing.

However, this simple rapid typing method is easily applicable in a common clinical virology laboratory, since it requires only widely available infrastructures and know-how (real time thermocycler and agarose gel electrophoresis devices). In comparison with recently published alternative methods pVI rapid typing is cheap and involves neither nested PCR, thus limiting the risk of laboratory contamination, nor array image analysis (Biere and Schweiger, 2010; Yliharsila et al., 2013). In addition including a size standard with species-specific pVI PCR products may facilitate species prediction in a patient sample for both melting curve analysis and size determination. This pVI rapid typing method, first designed and validated on selected adenoviral reference strains, has proven to be 100% reliable in identifying A and C species, the most relevant in our own hospital setting (Berciaud et al., 2012). It could easily be applied on a routine basis in several diagnosis laboratories, to improve virological monitoring in immunosuppressed and severely affected Adenovirus-infected patients.

Table 3
Prevalence of each Adenovirus serotype identified in the sample of patients with both the gold standard test (Sanger sequencing method) and the pVI test (n = 55). Estimations of sensitivity, specificity, positive and negative predictive values for the pVI test in the TYPADENO study.

Serotype	n (%)[a]		Sensitivity [CI95%]		Specificity [CI95%]		PPV [CI95%]		NPV [95%]	
A	4	(7.3)	100.0	[39.8–100.0]	100.0	[93.0–100.0]	100.0	[39.8–100.0]	100.0	[93.0–100.0]
B.	12	(21.8)	100.0	[73.5–100.0]	72.1	[56.3–84.7]	50.0	[29.1–70.9]	100.0	[88.8–100.0]
C	21	(38.2)	100.0	[83.9–100.0]	100.0	[89.7–100.0]	100.0	[83.9–100.0]	100.0	[89.7–100.0]
D	2	(3.6)	100.0	[15.8–100.0]	100.0	[93.3–100.0]	100.0	[15.8–100.0]	100.0	[93.3–100.0]
E	4	(7.3)	100.0	[39.8–100.0]	100.0	[93.0–100.0]	100.0	[39.8–100.0]	100.0	[93.0–100.0]
F	12	(21.8)	0.0	[00.0–3.5]	100.0	[91.8–100.0]	NA[b]		78.2	[65.0–88.2]
G	0	(0.0)	NA[c]		100.0	[93.5–100.0]	NA[c]		100.0	[93.5–100.0]

Notations: n = effective, % = rate in percent, CI95% = 95% Confidence Interval, PPV = Positive Predictive Value, NPV = Negative Predictive Value

[a] The prevalence was calculated using the results of the gold standard test (Sanger sequencing method).

[b] The PPV could not be calculated because no serotype F has been identified by pVI rapid typing test.

[c] Sensitivity and PPV could not be calculated because no serotype G was identified by both tests.

Funding

Appel d'offres interne CHU de Bordeaux 2011.

Conflict of interest

We are indebted in Pr Veronique Dubois for her kind help in analyzing bacteriological data.Competing interests: none declared

Ethical approval

obtained from the Comité de Protection des Personnes Sud Ouest et Outre Mer III on november 2011/06 (DC 2011/64).

Acknowledgements

Typadeno study members thank for their support Bordeaux University Hospital for its "Appel d'offres interne 2011" grant, CNRS UMR 5234 located in the Bordeaux University, Audrey Montero and Caroline Palacin who helped efficiently as clinical research associates.

References

Abbas, K.Z., Lombos, E., Duvvuri, V.R., Olsha, R., Higgins, R.R., Gubbay, J.B., 2013. Temporal changes in respiratory adenovirus serotypes circulating in the greater Toronto area, Ontario, during December 2008 to April 2010. Virol. J. 10, 15.

Baldwin, A., Kingman, H., Darville, M., Foot, A.B., Grier, D., Cornish, J.M., et al., 2000. Outcome and clinical course of 100 patients with adenovirus infection following bone marrow transplantation. Bone Marrow Transplant. 26 (December (12)), 1333–1338.

Berciaud, S., Rayne, F., Kassab, S., Jubert, C., Faure-Della Corte, M., Salin, F., et al., 2012. Adenovirus infections in Bordeaux University Hospital 2008–2010: clinical and virological features. J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol. 54 (August (4)), 302–307.

Biere, B., Schweiger, B., 2010. Human adenoviruses in respiratory infections: sequencing of the hexon hypervariable region reveals high sequence variability. J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol. 47 (April (4)), 366–371.

Cheng, J.L., Peng, C.-C., Chiu, N.-C., Weng, L.-C., Chiu, Y.-Y., Chang, L., et al., 2015. Risk factor analysis and molecular epidemiology of respiratory adenovirus infections among children in northern Taiwan, 2009–2013. J. Microbiol. Immunol. Infect. Wei Mian Yu Gan Ran Za Zhi.

Demian, P.N., Horton, K.C., Kajon, A., Siam, R., Hasanin, A.M.N., Elgohary Sheta, A., et al., 2014. Molecular identification of adenoviruses associated with respiratory infection in Egypt from 2003 to 2010. BMC Infect. Dis. 14, 50.

Echavarria, M., 2008. Adenoviruses in immunocompromised hosts. Clin. Microbiol. Rev. 21 (October (4)), 704–715.

Gray, G.C., McCarthy, T., Lebeck, M.G., Schnurr, D.P., Russell, K.L., Kajon, A.E., et al., 2007. Genotype prevalence and risk factors for severe clinical adenovirus infection, United States 2004–2006. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 45 (November (9)), 1120–1131.

Heim, A., Ebnet, C., Harste, G., Pring-Akerblom, P., 2003. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. J. Med. Virol. 70 (June (2)), 228–239.

Heim, A., 2011. Advances in the management of disseminated adenovirus disease in stem cell transplant recipients: impact of adenovirus load (DNAemia) testing. Expert Rev. Anti Infect. Ther. 9 (November (11)), 943–945.

Kalpoe, J.S., van der Heiden, P.L.J., Barge, R.M.Y., Houtzager, S., Lankester, A.C., van Tol, M.J.D., et al., 2007. Assessment of disseminated adenovirus infections using quantitative plasma PCR in adult allogeneic stem cell transplant recipients receiving reduced intensity or myeloablative conditioning. Eur. J. Haematol. 78 (April (4)), 314–321.

Kroes, A.C.M., de Klerk, E.P.A., Lankester, A.C., Malipaard, C., de Brouwer, C.S., Claas, E.C.J., et al., 2007. Sequential emergence of multiple adenovirus serotypes after pediatric stem cell transplantation. J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol. 38 (April (4)), 341–347.

Lai, C.-Y., Lee, C.-J., Lu, C.-Y., Lee, P.-I., Shao, P.-L., Wu, E.-T., et al., 2013. Adenovirus serotype 3 and 7 infection with acute respiratory failure in children in Taiwan, 2010–2011. PLoS One 8 (1), e53614.

Leruez-Ville, M., Minard, V., Lacaille, F., Buzyn, A., Abachin, E., Blanche, S., et al., 2004. Real-time blood plasma polymerase chain reaction for management of disseminated adenovirus infection. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 38 (January (1)), 45–52.

Leruez-Ville, M., Chardin-Ouachee, M., Neven, B., Picard, C., Le Guinche, I., Fischer, A., et al., 2006. Description of an adenovirus A31 outbreak in a paediatric haematology unit. Bone Marrow Transplant. 38 (July (1)), 23–28.

Li, Y., Zhou, W., Zhao, Y., Wang, Y., Xie, Z., Lou, Y., et al., 2015. Molecular typing and epidemiology profiles of human adenovirus infection among paediatric patients with severe acute respiratory infection in China. PLoS One 10 (4), e0123234.

Lindemans, C.A., Leen, A.M., Boelens, J.J., 2010. How I treat adenovirus in hematopoietic stem cell transplant recipients. Blood 116 (December (25)), 5476–5485.

Lion, T., Baumgartinger, R., Watzinger, F., Matthes-Martin, S., Suda, M., Preuner, S., et al., 2003. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. Blood 102 (August (3)), 1114–1120.

Lion, T., Kosulin, K., Landlinger, C., Rauch, M., Preuner, S., Jugovic, D., et al., 2010. Monitoring of adenovirus load in stool by real-time PCR permits early detection of impending invasive infection in patients after allogeneic stem cell transplantation. Leukemia 24 (April (4)), 706–714.

Madisch, I., Wolfel, R., Harste, G., Pommer, H., Heim, A., 2006. Molecular identification of adenovirus sequences: a rapid scheme for early typing of human adenoviruses in diagnostic samples of immunocompetent and immunodeficient patients. J. Med. Virol. 78 (September (9)), 1210–1217.

Qurei, L., Seto, D., Salah, Z., Azzeh, M., 2012. A molecular epidemiology survey of respiratory adenoviruses circulating in children residing in Southern Palestine. PLoS One 7 (8), e42732.

Sarantis, H., Johnson, G., Brown, M., Petric, M., Tellier, R., 2004. Comprehensive detection and serotyping of human adenoviruses by PCR and sequencing. J. Clin. Microbiol. 42 (September (9)), 3963–3969.

Sriwanna, P., Chieochansin, T., Vuthitanachot, C., Vuthitanachot, V., Theamboonlers, A., Poovorawan, Y., 2013. Molecular characterization of human adenovirus infection in Thailand, 2009–2012. Virol. J. 10, 193.

Walls, T., Shankar, A.G., Shingadia, D., 2003. Adenovirus: an increasingly important pathogen in paediatric bone marrow transplant patients. Lancet Infect. Dis. 3 (February (2)), 79–86.

Wang, W., Cavailler, P., Ren, P., Zhang, J., Dong, W., Yan, H., et al., 2010. Molecular monitoring of causative viruses in child acute respiratory infection in endemo-epidemic situations in Shanghai. J. Clin. Virol. Off. Publ. Pan. Am. Soc. Clin. Virol. 49 (November (3)), 211–218.

Wang, H., Zheng, Y., Deng, J., Chen, X., Liu, P., Li, X., 2015. Molecular epidemiology of respiratory adenovirus detection in hospitalized children in Shenzhen, China. Int. J. Clin. Exp. Med. 8 (9), 15011–15017.

Wurzel, D.F., Mackay, I.M., Marchant, J.M., Wang, C.Y.T., Yerkovich, S.T., Upham, J.W., et al., 2014. Adenovirus species C is associated with chronic suppurative lung diseases in children. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 59 (July (1)), 34–40.

Yamamoto, D., Okamoto, M., Lupisan, S., Suzuki, A., Saito, M., Tamaki, R., et al., 2014. Impact of human adenovirus serotype 7 in hospitalized children with severe fatal pneumonia in the Philippines. Jpn. J. Infect. Dis. 67 (2), 105–110.

Yliharsila, M., Harju, E., Arppe, R., Hattara, L., Holsa, J., Saviranta, P., et al., 2013. Genotyping of clinically relevant human adenoviruses by array-in-well hybridization assay. Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 19 (June (6)), 551–557.

Yun, H.C., Young, A.N., Caballero, M.Y., Lott, L., Cropper, T.L., Murray, C.K., 2015. Changes in clinical presentation and epidemiology of respiratory pathogens associated with acute respiratory illness in military trainees after reintroduction of adenovirus vaccine. Open Forum Infect. Dis. 2 (September (3)), ofv120.