PCR for crypto-infections diagnosis in patients with PTLDS

Comparison of matrices (venous blood, capillary blood, urine and saliva), effect of a biofilm breaker (serrapeptase)

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Pathotique 2 Study

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  * **Bionops, France** (which provided serrapeptase)

PCR

* Various techniques, often home-made
* Various results
* Various matrices

* Frequent low sensitivity

* Need for comparative studies and standardization
* Need for a collaboration between human labs and vet labs

Pathotique 2 Study
Methods (1)

* **PCR realized for 18 bacteria, 1 fungus** (*Candida*), **2 parasites** (*Babesia* & *Theileria*) **and 13 viruses**

* **Patients with SPPT** (persistent polymorphic syndrome after a possible tick bite, syndrome recognized by the French High Authority for Health, HAS), **close to PTLDS** (post-treatment Lyme disease syndrome)

* Not treated with anti-infective drugs for at least 2 months

Pathotique 2 Study Methods (2)

* **PCR**

* **Two samples from each matrix, at Day 0 and Day 3**
  * Venous blood
  * Capillary blood
  * Urine
  * Saliva

* **After the first sample, intake per os of 480 000 IU of serrapeptase** (biofilm breaker, *Bionops*) **during 3 days**

Pathotique 2 Study Methods (3)

* **PCR**

* **ADNucleis** extraction buffer (5M guanidium thiocyanate, 500 mM TrisHCL, 50 mM EDTA, 20% Tween 20, 20% Triton X-100, 750 µg proteinase K.

* Extraction on 300 µl of venous blood, capillary blood, saliva and urine pellet from 10 ml centrifuged urine

Pathotique 2 Study Methods (4)

* **Quantitative PCR**
  * 12 µl of extracted DNA or RNA
  * Primers and probes from reference laboratories or articles (since 2000)
  * ADNucleis PCR buffer (20mM Tris-HCl, 10 mM NH_4SO_4, 10mM KCl, 2 mM Mg^{2+}, 0.1% TritonX-100, pH 8.8), 2 mM of each dNTP, 600 nM of each primer, 1 µl of Evagreen and 5 units of *Taq* polymerase ADNucleis
  * Taqman or Sybr Technology
  * Synthetised DNA sequence insert in a plasmid is used as positive control
  * PCR program : Initial denaturation step of 5 min at 95°C followed by 42 cycles of 15 s at 95°C and 40 s at 60°C (hybridization-elongation) and a last step of dissociation (10 min with temperature increments from 75°C to 95°C)
  * Quantification with serial dilution of the positive control

Pathotique 2 Study Methods (5)

* For ***Borrelia burgdorferi sensu lato* species** detection, three kits were used:
  * *B. burgdorferi s.l.*
  * *B. afzelii*
  * *B. garinii*
  * These last two kits are more sensitive

Babesia / Theileria

* **In 2006 *Babesia microti* was renamed *Theileria microti*, thanks to the sequencing and the comparison of its ribosomal RNA.**
  * Uilenberg,G. Goff,W.L. (2006). "Polyphasic Taxonomy". Annals of the New York Academy of Sciences. 1081 (1): 492–7.
  * Uilenberg, G (May 2006). "*Babesia*--a historical overview". Veterinary Parasitology. 138 (1–2): 3–10.

* **In our study, *Theileria microti* was detected with *Theileria* spp primers**, not with *Babesia* primers

* ***Babesia spp* primers correspond to species of *Babesia* not yet identified** (possibly *B. capreoli, B. duncani, B. divergens, B. venatorum* or *B. odocoilei*).

Babesia / Theileria

* **Current studies are trying to identify species.** With the following sequences:

```
B.spp ACCTGCTAACTAGTGTCCGTAAAAAGGTTCGTCC.GTTACGGTTTGCTTCTTAGAGGGACTTTGCGGCTCTAAGCCGCAAGGAAGTTTAAGGCAATAACAGGTCTGTG
B.div ACCTGCTAACTAGTGTCCGTAAAAAGGTTCGTCC.GTTACGGTTTGCTTCTTAGAGGGACTTTGCGGCTCTAAGCCGCAAGGAAGTTTAAGGCAATAACAGGTCTGTG
B.cap ACCTGCTAACTAGTGTCCGTAAAAAGGTTCGTCC.GTTACGGTTTGCTTCTTAGAGGGACTTTGCGGCTCTAAGCCGCAAGGAAGTTTAAGGCAATAACAGGTCTGTG
B.ven  ACCTGCTAACTAGTGTCCGTAAAAAGGTTCGTCC.GTTACGGTTTGCTTCTTAGAGGGACTTTGCGGCTCTAAGCCGCAAGGAAGTTTAAGGCAATAACAGGTCTGTG
B.bov* ACCT..TAACCTGCTATTTAGTCGCTCGGTCTCT.GTCCGTGCGCACTTCATAGAGGGACTCTGCGGCGTCAAGCTGCGGTGAGGTTTAAGGCAATAACAGGTCTGTG
B.mic* ACCT..TAACCTGCTAACTAGTTGCCGTTATTTCAGTTTCGGCCAGCTTCTTAGAGGGACTTTGGGGCTCTAAGCCACAAGGAAGTTTAAGGCAATAACAGGTCTGTG
T.equ* ACCTGCTAAATAGGGT..GTTGG.A.GTTAT....GTTCTACACTGCTTCTTAGAGGGACTTTGCGGTCATAAATCGCAAGGAAGTTTAAGGCAATAACAGGTCTGTG
```

Results of PCR in Pathotique 2 Study

* 105 patients
* PCR positive for at least one microbe
*
  * Venous blood: 20 (19%)
  * Capillary blood: 53 (50.5%)
  * Urine: 65 (61.9%)
  * Saliva: 60 (57.1%)

Comparison of the results of PCR in Pathotique 1 and Pathotique 2 Studies

* **PCR positive for at least one microbe:**

	Study 1	*Study 2*
* **Venous blood:**	**12/71 (17%)**	**20/105 (19%)**
* **Capillary blood:**	**15/34 (45%)**	**53/105 (50.5%)**
* **Urine:**	**34/71 (48%)**	**65/105 (61.9%)**
* **Saliva:**	**64/71 (90%)**	**60/105 (57.1%)**

Results of PCR for bacteria and *Candida*. All matrices (venous blood, capillary blood, urine & saliva)

- **High frequency ≥ 29%**
  - *Mycoplasma spp* 97 (92.4%)
  - *Candida spp* 33 (31.4%)
  - *Rickettsia spp* 31 (29.5%)

- **Moderate frequency between 6% and 12%**
  - *Bartonella spp* 12 (11.4%)
  - *Bartonella henselae* 11 (10.5%)
  - *Borrelia garinii* 7 (6.7%)
  - *Ehrlichia spp* 7 (6.7%)
  - *Chlamydia spp* 7 (6.7%)

Results of PCR for bacteria. All matrices (venous blood, capillary blood, urine & saliva)

- **Low frequency < 2%**
  - *Brucella spp* 2 (1.9%)
  - *Bartonella quintana* 2 (1.9%)
  - *Borrelia afzelii* 2 (1.9%)
  - *Borrelia burgdorferi s.l.* 2 (1.9%)
  - *Borrelia hermsii* 2 (1.9%)
  - *Coxiella burnetii* 1 (1%)
  - *Neoehrlichia mikurensis* 1 (1%)

- **Not isolated in this series**
  - *Borrelia miyamotoi*
  - *Francisella tularensis*
  - *Anaplasma spp*

Borrelia miyamotoi: 43 cases diagnosed in France by real-time PCR in patients with persistent polymorphic signs and symptoms.

M. Franck et al. Front Med 2020.

doi.org/10.3389/fmed.2020.00055

* **824 patients tested: 43 (5.22%) positive for *B. miyamotoi***

* **In the present study, 105 patients tested: no *Borrelia miyamotoi***
  * *This difference is not significant (frequency 0 is within the fluctuation interval which ranges from -0.05 to +0.15, with a threshold of 95%*

Results of PCR for parasites. All matrices (venous blood, capillary blood, urine & saliva)

* ***Theileria spp*** **30 (28.6%)**
* ***Babesia spp*** **3 (2.9%)**

Results of PCR for viruses. All matrices (venous blood, capillary blood, urine & saliva)

* CMV	3 (2.9%)
* HHV-6	3 (2.9%)
* Chikungunya	2 (1.9%)
* Zika	1 (1%)
* West Nile	1 (1%)
* TBEV	0
* EBV	0
* VZV	0
* Dengue	0
* Bourbon	0
* Powassan	0
* Eyach	0

Results of PCR for bacteria and *Candida*. Positive matrices (venous blood, capillary blood, urine & saliva)

* **High frequency ≥ 25%**

		Venous blood	*Capillary blood*	*Urine*	*Saliva*
* ***Mycoplasma spp***	**97**	3	17	50	96
* ***Candida spp***	**33**	7	12	15	1
* ***Rickettsia spp***	**31**	1	2	16	29

Results of PCR for bacteria. Positive matrices (venous blood, capillary blood, urine & saliva)

* **Moderate frequency between 5% and 24%**

		Venous blood	*Capillary blood*	*Urine*	*Saliva*
* ***Bartonella spp***	**12**	5	5	2	4
* ***Bartonella henselae***	***11***	3	2	6	*1*
* ***Borrelia garinii***	**7**			1	6
* ***Ehrlichia spp***	**7**	2	4	1	2
* ***Chlamydia spp***	**7**				8

Results of PCR for bacteria. Positive matrices (venous blood, capillary blood, urine & saliva)

* **Low frequency < 5%**

		Venous blood	*Capillary blood*	*Urine*	*Saliva*
* ***Brucella spp***	**2**	1			1
* ***Bartonella quintana***	**2**			1	1
* ***Borrelia afzelii***	**2**		1	1	1
* ***Borrelia burgdorferi s.l.***	**2**		1	1	
* ***Borrelia hermsii***	**2**			1	1
* ***Coxiella burnetii***	**1**			1	
* ***Neoehrlichia mikurensis***	**1**		1		

Results of PCR for parasites. Positive matrices (venous blood, capillary blood, urine & saliva)

		Venous blood	*Capillary blood*	*Urine*	*Saliva*
* ***Theileria***	**30**	4	21	3	17
* ***Babesia***	**3**	1		1	1

Results of PCR for viruses. Positive matrices (venous blood, capillary blood, urine & saliva)

		Venous blood	*Capillary blood*	*Urine*	*Saliva*
* **CMV**	**3**		1	2	3
* **HHV-6**	**3**		1	1	1
* **West Nile**	**1**			1	
* **Chikungunya**	**2**			2	
* **Zika**	**1**			1	

PCR: 1st sample negative, 2nd sample positive at day 3 after serrapeptase

	Patients
* **Venous blood**	**9**
* **Capillary blood**	**16**
* **Urine**	**19**
* **Saliva**	**26**

PCR: Microbes only found in one matrix

* Venous blood	7
* Capillary blood	14
* Urine	24
* Saliva	71

PCR: Microbes only found in venous blood

* **7 patients**

* *Bartonella spp*	2
* *Bartonella henselae*	2
* *Rickettsia*	1
* *Babesia*	1
* *Theileria*	1

PCR: Microbes only found in capillary blood

* **14 patients**

* *Bartonella spp*	*1*
* *Ehrlichia*	*1*
* *B. burgdorferi s.l.*	*1*
* *Candida*	4
* *Theileria*	6
* HHV-6	1

PCR: Microbes only found in urine

* **24 patients**

* *Rickettsia*	*4*
* *Bartonella spp*	*2*
* *Bartonella henselae*	*3*
* *Bartonella quintana*	*1*
* *Ehrlichia*	*1*
* *Coxiella burnetii*	*1*
* *B. burgdorferi s.l.*	*1*
* *B. hermsii*	*1*
* *Candida*	6
* *Theileria*	1
* HHV-6	1
* Zika	1
* Chikungunya	1
* West Nile	1

PCR: Microbes only found in saliva

- **71 patients**

Rickettsia	4
Bartonella spp	2
Bartonella henselae	3
Bartonella quintana	1
Ehrlichia	1
Coxiella burnetii	1
B. burgdorferi s.l.	1
B. hermsii	1
Candida	6
Theileria	1
HHV-6	1
Zika	1
Chikungunya	1
West Nile	1

Salivary infection

External colonization or secretion from salivary glands?

* Tropism of some micro-organisms for salivary glands
* Role of saliva for transmission of some infections (e.g. rabies, EBV).
* Salivary glands are holomerocrine: secretion needs disruption of the apex of the acini cells.
* Thus, these acini cells must multiply rapidly. This could enhance the tropism of some micro-organisms.

PCRs: Comments

* ***Mycoplasma.*** High level of carriage in saliva and infection or colonization in urine (from the genital tract?). Superiority of capillary blood.
* ***Candida***: Superiority of capillary blood. Saliva: probable colonization. Urine: possible contamination during urine collection
* ***Bartonella***: Interest of capillary blood. Saliva: source of transmission?
* ***Borrelia:*** Low sensitivity
* ***Ehrlichia:*** Interest of capillary blood
* ***Theileria***: Superiority of capillary blood, combined with venous blood. Interest of serrapeptase

Conclusion
SPPT / PTLDS

* ***PCRs, interest to:***
  * ***Search a wide range of micro-organisms***
  * ***Take samples from several matrices (venous blood, capillary blood, urine and saliva)***
  * ***Take samples twice (serrapeptase)***

* ***An accurate microbial diagnosis may allow correlations with some signs and symptoms***