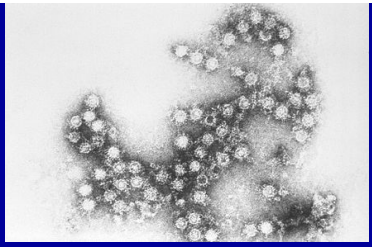
Enterovirus Infections and ME



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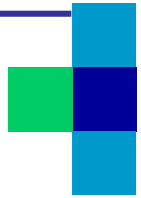
Enteroviruses: 111 serotypes

Virus	Serotypes	mouse pathogenicity
Poliovirus	1 - 3	-
Coxsackie virus A	1 - 22, 24	+
Coxsackie virus B	1 - 6	
Echovirus	1-7,9,11-27,29-33	-
Enterovirus	68 - 71	



Coxsackieviruses and CFS

- Since 1989 manuscripts discuss an association of entero-/ coxsackievirus infections with with ME/CFS
- Pubmed search:
  - 7796 papers: search CFS and ME in all fields
  - 81 papers with entero-/coxsackievirus in all fields
- Most studies: serology or PCR tests from stool, blood or muscle biopsies
- However, several studies did not confirm this findings
- Since 2010 interest in enteroviruses decreased: last publication 2011

Cunningham L et al, J Gen Virol 1990

- Muscle biopsies: in situ hybridization of enteroviral RNA
- 4 CFS patients positive
- Negative all 152 control samples of human muscle
  (108 normal biopsy samples,28 pathological biopsy samples including 24 inclusion body myositis;
- Persistence of enteroviral RNA in chronic fatigue syndrome is associated with abnormal production of equal amounts of positive and negative strands of enteroviral RNA

Coxsackie viruses and ME/CFS

- **Gow JW et al, Br. Med. Bull 1991**
  - **Panenterovirus - PCR**
    - **muscle: 53 % of CFS , 6.7 * more frequent than in controls**
    - **leukocytes in blood: 16% of patients and controls**
    - **serology: no difference**

Coxsackieviren und Chronic Fatigue

- **Nairn et al, J Med Virol. 1995 (Glasgow)**
  - **100 CFS and 100 controls**
  - **PCR: 42 % of CFS and 9% of controls**
  - **Neutralisation assay no difference: 34% CFS, 41% Kontrollen**
  - **Seronegative PCR+ patients**

- **Bowles et al. J Med 1993 (London)**
  - **In situ Hybridisierung mit Entervirus-Probe im Muskel**
    - **25/96 Patienten mit Myositis (DM, PM)**
    - **41/158 Patienten mit Postviralem Müdigkeitssyndrom**
    - **2/152 Kontrollen**

Coxsackieviren und Chronic Fatigue

Clements et al. , J Med Virol 1995

- Nested PCR
- enteroviral specific sequences in 36 of 88 serum samples from chronic fatigue patients
- 22 of 82 acutely ill individuals
- 3 of 126 healthy individuals

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Lane et al. J Neurol Neurosurg Psychiatry 2002

- Muscle biopsy samples from 20.8% of the CFS patients were positive for enterovirus sequences by RT-nPCR (most closely related to coxsackie B virus)
- 29 control samples: all negative; 0% positive
- 58.3% of the CFS patients had a SATET+response: abnormal lactate responses to exercise in the subanaerobic threshold exercise test.
- Nine of the 10 enterovirus positive cases were among the 28 SATET+ patients (32.1%),
- only one (5%) of the 20 SATET-negative patients was ev+.

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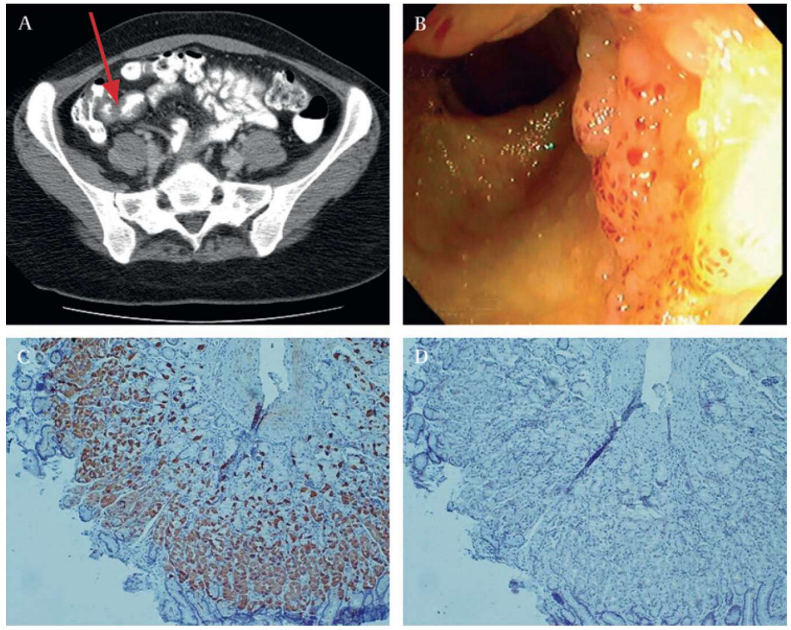
Coxsackie viruses and Fibromyalgia

- **Wittrup I.H. J Rheumatology 2001**
  - **50% with acute fibromyalgia show IgG against Enteroviruses**
  - **15 % of slow-onset-patients**

Acute enterovirus infection in 3 patients developing CFS with persistent EV detection

- Chia JK. Acute enterovirus infection followed by myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and viral persistence
- J Clin Pathol 2010



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- Chia J, et al.. Chronic fatigue syndrome is associated with chronic enterovirus infection of the stomach. J Clin Pathol 2008

- 135/165 (82%) stomach biopsies from CFS patients stained positive for VP1 within parietal cells,
- only 7/34 (20%) of the controls ($p<0.001$).

- Biopsies taken from six patients at the onset of the CFS with abdominal symptoms, and 2–8 years later showed positive staining in the paired specimens.

- EV RNA was detected in 9/24 (37%) paraffin embedded biopsy samples; 1/21 controls had detectable EV RNA ($p<0.01$);

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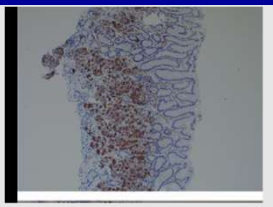

Table 1 Virologic analysis of stomach samples from patients with chronic fatigue syndrome (CFS)

				BGMK cell culture					
			RNA-later sp.	Normal cells	5-idu, dex-treated cells				
				Primary culture*				1st pass†	
Patient no	No years sick	VP1 stain	EV RNA	4 wk	4 wk	6 wk	8 wk	4 wk	8 wk
1	5	–	–	–	++	+++	–	+++	–
2	4	nd	nd	–	–	+++	–	++	–
3	6	++	+	–	–	+++	+++	+	–
4	10	++	+	–	++	++	–	+++	–
5	12	++	+	–	–	++	–	++	–
6	4	+	–	–	–	+	–	nd	–
7	8	–	nd	–	+++	++	++	+	–
8	6	nd	–	–	–	+	–	–	–
9	10	+	+++	–	+	++	++	++	–
10	13	+	–	–	+	nd	nd	nd	nd
11	3	++	++	–	–	nd	nd	nd	nd
12	15	++	++	–	–	nd	nd	nd	nd
13	20	–	–	–	–	nd	nd	nd	nd

5-idu, 5-idoxyuridine; dex, dexamethasone; EV, enterovirus; VP1, viral capsid protein 1.
Three to four biopsies were obtained from each patient's antrum and processed for VP1 staining, RNA assay and viral culture. VP1 staining was graded as described in methods. EV RNA was determined on the RNA-later specimens and culture lysates at periods indicated. The optical density reading of the RT-PCR ELISA was rated as +, if >1.0, <1.9; ++, if >2.0, <3.0; +++, if ⩾3.0; nd, not done.
*The tissue homogenates were inoculated onto untreated BGMK cells (normal) or 5-idu- and dex-treated BGMK cells; half of the tissue culture monolayers were scraped and lysed and EV RNA determined at the time period indicated.
†Lysates from the 6-week-old primary culture were passed to another set of uninfected, 5-idu- and dex-treated cells, and the presence of EV RNA was assayed at the time period indicated.

Negative Studies for Enteroviruses and CFS

- **Gow et al. CID 1994**
- RT-PCR in muscle biopsies
- positive in CFS patients: 32 (26.4%)
- controls: patients with other neurological diseases: 20 (19.8%)
- difference was not significant

- Had previously published a positive association in 1991 in Br Med Bull.

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Negative Studies for Enteroviruses and CFS

- **McArdle et al. Clin Science 1996**
- RT-PCR in muscle biopsies
- negative in 34 CFS patients

- **Lindh et al. Scand J Infect Dis 1996**
- 34 Swedish patients
  - negative stool cultures in 25 patients
  - negative paired enterovirus specific IgG serum-CSF samples
  - negative PCR in muscle biopsies of 29 patients

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Negative Studies for Enteroviruses and CFS

- **Swanink et al. CID 1994**
- 76 patients with disabling unexplained fatigue and 76 matched controls
- No difference by serological or antigen testing.
- no enteroviruses were isolated from any stool culture.
- Enterovirus was detected by PCR in one stool specimen from a patient with CFS but was not detectable in a second sample obtained from the same patient 3 months later.
- All stool specimens from controls were PCR-negative.

Patient G.M.

- 38 y old male nurse in cardiology
- 1997 Coxsackie B5 infection: NT-titer: B5: 1:1280,
  duration of disease 6 months
- 5/98 when asymptomatic control of NT titer: 1:160
- 10/1998 new symptoms: myalga, predominantly back muscles, strong weakness, fatigue
- B5-Titer: increase to 1:640
- Immunglobulins: Intraglobin 5 g, then 10 g,
- Remission for 3 years

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Patient GM

- antibody titer in remission: 9/99 1:80
- 12/2001: relapse with myalgias, myogelosis in back muscles
- strong fatigue
- Coxsackie virus titer: 1:640
- PCR from mouth rinse: positive for Coxsackievirus B5
- Except for slight elevation of IgA normal lab. (CD4/CD8 2.7)
- Intravenous immunoglobulins: Intraglobin: 1 * 10g , 1 * 5 g
- Since then in remission except for slight rare myalgias

Erlangen Coxsackie Virus Cohort

- 22 patients with very high NT-Titer >= 1:1280
- Such high titers indicate active infection, post-infection titers usually 1:40 to 1:160
- 4 male and 18 females
- Mean age: 40 years (standard deviation 8 y)
- Symptoms:
  - CFS
  - myalgia or arthralgia

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Erlanger Coxsackie Cohort:

- **NT-titers : ≥1:640 until ≥2560**
- **Cox B2: 36%, B5 36%, B1,3,4: 7% each**
- **Many patients show antibody titer against one B variant and lower titers against some other variants (B2/B5)**
- **Unclear whether cross-reaction of separate infections**
- **indications that antibodies against one serotype can enhance infection against other serotypes**

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Coxsackie Virus Detection

- PCR positive in mouth rinse in 1 patient (B5)
- Virus culture from mouth rinse: 1 patient (B4)
- Detection in stool by PCR in 2 cases
- Virus isolation from stool: no patient,

Disease onset

- Defined sudden onset: 14/22 patients
- 10 of 22 patients flu-like symptoms
- 2 patients with diarrhoea
- 1 patient gastrointestinal pain and elevated liver enzymes
- 1 patient with arthrits
- 8/22 patients slow development without defined begin.

Potential mechanisms of CFS induction by Coxsackie viruses

- **viral persistence**
  - **direct viral damage of muscles and nerves/brain**
- **immunodeficiency:**
  - **lack of an innate antiviral defense**
  - **continuous immunaktiviation with local cytokine release**
- **autoimmunity:**
  - **chronic immune stimulation leads to formation of autoantibodies**

Erlanger Coxsackie Cohort: typical symptoms

- **absolute loss of energy, exhaustion aggravated by exercise**
- **high sleep requirement without recovery**
- **diffuse mental impairment**
- **normal appetite, frequently increased weight**
- **frequently slight gastrointestinal complaints, rarely diarrhoea**
- **myalgia: diffus, no pressure pain, no tenderpoints,**
- **arthralgia: multiple joints, rare arthritis**
- **secondary depression**

Laboratory

- Elevation of liver enzymes
  - GOT: 14%
  - GPT: 59%
  - Gamma-GT: 36%

- 3 Patienten showed Borrelia specific antibodies: IgM and IgG. pos.

  no improvement by antibiotic therapy

Course of Disease

- All patients had strong impairment of well-being, daily life and on their work situation.
- All patients were not able to work, most several weeks, some over years
- No complete recovery
- Many experienced problems in partnership and marriage
- seconday psychiatric symptoms: depression

Summary

- Conflicting results in literature regarding role of enterovirus / coxsackieviruses in CFS/ME
- Own data indicate a role of Coxsackieviruses in a subgroup of CFS patients.
- Persistent infection < hit and run – autoimmunity ?
- Decisive test: neutralisation antibody titer
- more research needed!

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Enteroviruses

- Picorna viruses (Pico RNA - virus)
- acid resistant (pH 3 -5 for 1 - 3 h)
- resistant against many detergents and disinfectants
  - soap, chloroform
- stable at 4 degree Celsius over weeks

  at room temperature over days
- Inactivation by drying, uv light, heat, formaline, hypochlorite:

Coxsackie Viruses

- 1948 discovered by Dalldorf and Sickles
- Inoculation of baby mice with fecal suspension of two patients with suspicion of polio:
  - Paralysis of mice by this new virus
  - Name of the virus according to the home town of patients: Coxsackie, New York
  - thereafter isolation of several viral variants:
    - flaccid paralysis: Group A: generalized Myositis
    - spastic paralysis: Group B: degenerative Plaques in brain, muscle and pankreas, only focal lesions in muscles

Coxsackie Viruses

- **Genome: a single-stranded +sense RNA molecule**
- **RNA is infectious**
- **Replication only in cytoplasm (glattes ER)**
- **Replication also in enucleated cells**
- **Duration of replication cycle in 5 - 10 hours**
- **genomic RNA diretly translated by polysomes**
- **after 30 minutes complete inhibition of cellular protein synthesis by cleavage of the cap-binding-complex on eukaryotic mRNA.**

Coxsackie Viruses

- cytopathic virus: lethal for newborn mice
- direct damage by tissure destruction
- In addition immunopathology: rash, myocarditis
- replication:
  - primary replication: epithelial cells of gastrointestinal und respiratory tract
  - viremia
  - secundary replication: CNS, muscles, heart muscle
- persistence in heart muscle
- specific receptor:
  - HCAR (human coxsackie virus and adenovirus receptor)
  - on many cells, especially epithelial cells)

Enteroviruses: Epidemiology

- highest concentration in stool
- isolation also from respiratory secretions
- transmission: fecal/oral and saliva / large droplets
- epidemic and endemic emergence
  - B5: US-epidemics 1961, 1967, 1972, 1983
  - B2,B4: annular in similar frequencies
- isolation of multiple variants from one patient
- high prevalence, 4 - 6 % of population are IgM+

Coxsackie Viruses

- **Flu like symptoms**
- **aseptic meningitis, encephalitis (typ A9)**
- **rash: herpangina, hand-foot-mouth-disease (A serotypes, A16)**
- **mild respiratoric syndromes, pharyngitis**
- **pleurodynia, Morbus Bornholm (B serotypes)**
- **myocarditis (B5), pericarditis**
- **in newborns hepatitis**
- **hypothesized associations:**
  - **Diabetes mellitus ? (B4)**
  - **fetopathies**
  - **chronic fatigue syndrome**

Enteroviruses

- **Life long protection after infection with one individual serotypes**
- **No or low cross-protection against other serotypes**
- **strong humoral immune response (IgG 1 and 3)**
- **strong variations in neutralizing domains**
  - **also within a variant: Coxsackie B4**
- **immune response heterotypic**
- **neutralization: serotypic**

Coxsackieviruses: Diagnosis

- **serology:**
  - **KBR: IgM (not serotype specific)**
  - **ELISA: IgG, IgM**
  - **Neutralization test**
- **virus isolation: cell culture**
- **PCR: pan-picorna virus PCR, pan-enterovirus PCR:**

  stool, mouth rinse, CSF, serum, biopsies
- **in-situ-hybridizytion**
- **staining of cells with VP1 antibody**

Coxsackie virus: treatment

- **Pleconaril:**
  - **active against many picorna viruses, also Coxsackie virus**
  - **Virapharm, USA**
  - **Compassionate use in enterovirus encephalitis**
  - **No effect in patient with B4 infection**
- **immunglobulines: single cases, CVID**
- **ribavirin in animal models partially active**
- **Interferons: case reports**
- **Fluoxetin**