22. TÜRK KLİNİK MİKROBİYOLOJİ VE İNFEKSİYON HASTALIKLARI KONGRESİ

KLİMİK 2022

Mikrobiyolojik Tanıda Sendromik Paneller Ülkemiz Koşullarında Ne Kadar Yararlı?

Santral Sinir Sistemi İnfeksiyonları

DOÇ. DR. ULULHAN SİLİ

MARMARA ÜNİVERSİTESİ TIP FAKÜLTESİ

İNFEKSİYON HASTALIKLARI VE KLİNİK MİKROBİYOLOJİ AD

12 MART 2022, SİMPOZYUM 30

MARMARA ÜNİVERSİTESİ 1883 | Tıp Fakültesi

Santral Sinir Sistemi (SSS) İnfeksiyonları

- SSS infeksiyonlarının tanısında sendromik panelin yeri ve önemi

- Sendromik Panel
  - >1'den fazla patojenin genomik materyalinin PCR ile saptanması
  - belli bir sendroma neden olan en sık patojenlerin testinin 1 panele sığdırılması (Fox AS el al. 2021)

- Potansiyel faydaları
  1. Etkeni kısa sürede belirleyerek doğru tedaviye başlama
  2. Etkeni kısa sürede belirleyerek veya infeksiyoz etiyolojiyi dışlayarak gereksiz geniş spektrumlu antimikrobiyal maruziyetini önleme

- Rehberler ne öneriyor?

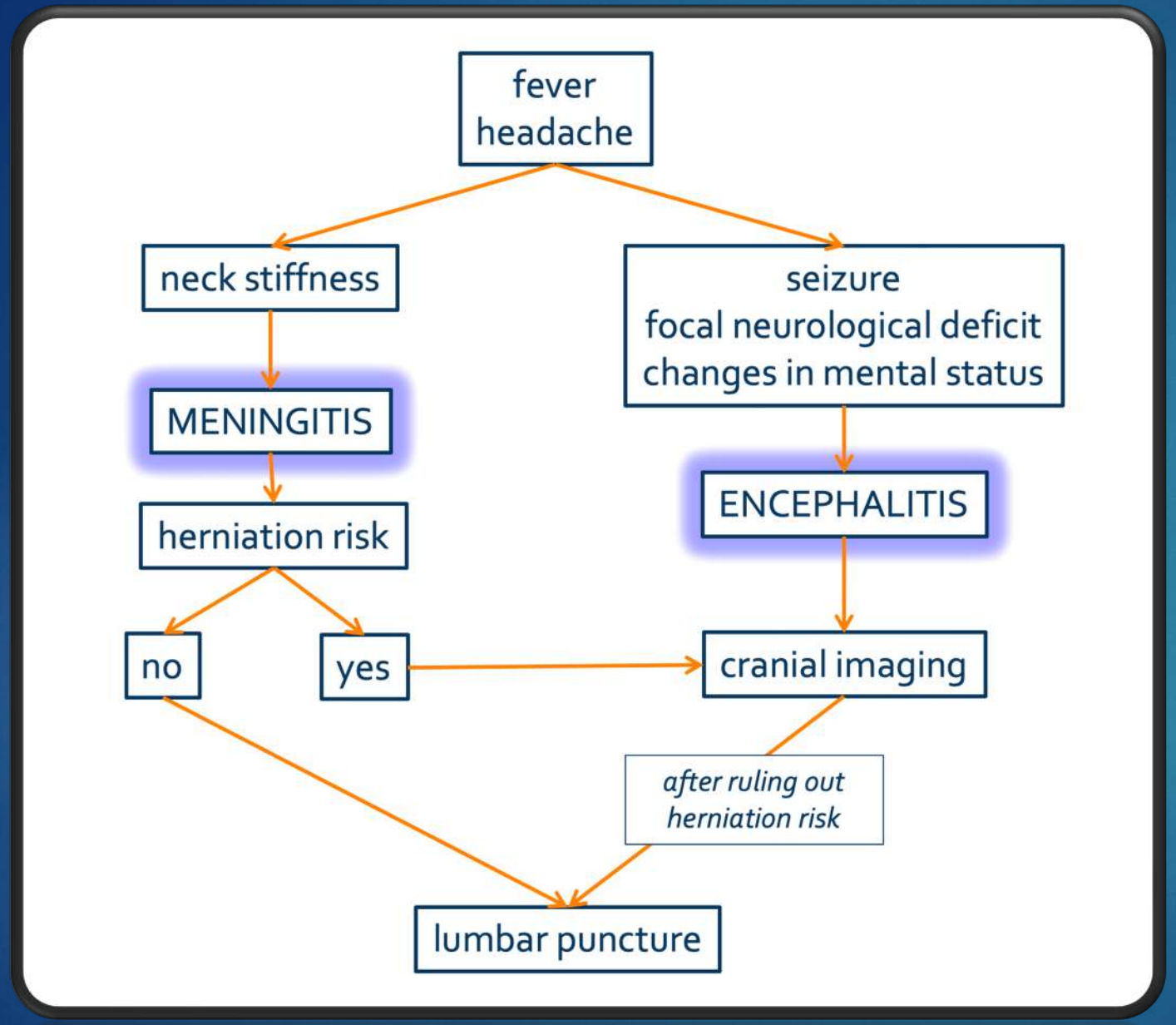
Santral Sinir Sistemi (SSS) İnfeksiyonları

- Fox AS el al. 2021 Syndromic testing for the diagnosis of infectious diseases: the right test if used for the right patient
  "İnfeksiyon hastalıklarının tanısı için sendromik testler: **Doğru hasta için kullanıldığında doğru test**"
- "Bu moleküler testlerin mevcut olması ve enfeksiyonların teşhisinde önemli bir rol oynaması, bunların **mutlaka kullanılacağı veya kullanılması gerektiği anlamına gelmiyor**"
- "Antimikrobiyal yönetimine benzer şekilde, **tanısal test yönetimi** de klinik sonuçları optimize ederek ve komplikasyonları en aza indirerek hasta yönetimine rehberlik etmek için **tüm laboratuvar testlerinin uygun şekilde** kullanılmasını ifade eder"

- Ülkemiz koşullarında kullanımı, yararı?

Katılımcılara sorular

- SSS infeksiyonlarının tanısında sendromik panel **merkezinizde kullanılıyor mu**?
  Bu teste ulaşabiliyor musunuz?
- Sizce SSS infekiyonlarının tanısında sendromik panel **mutlaka** kullanılmalımıdır?
- SSS infeksiyonlarının tanısında **geleneksel yöntemler yeterlidir**?
  Gram, kültür, antijen, seroloji ve tekli PCR (low-plex/ in-house)

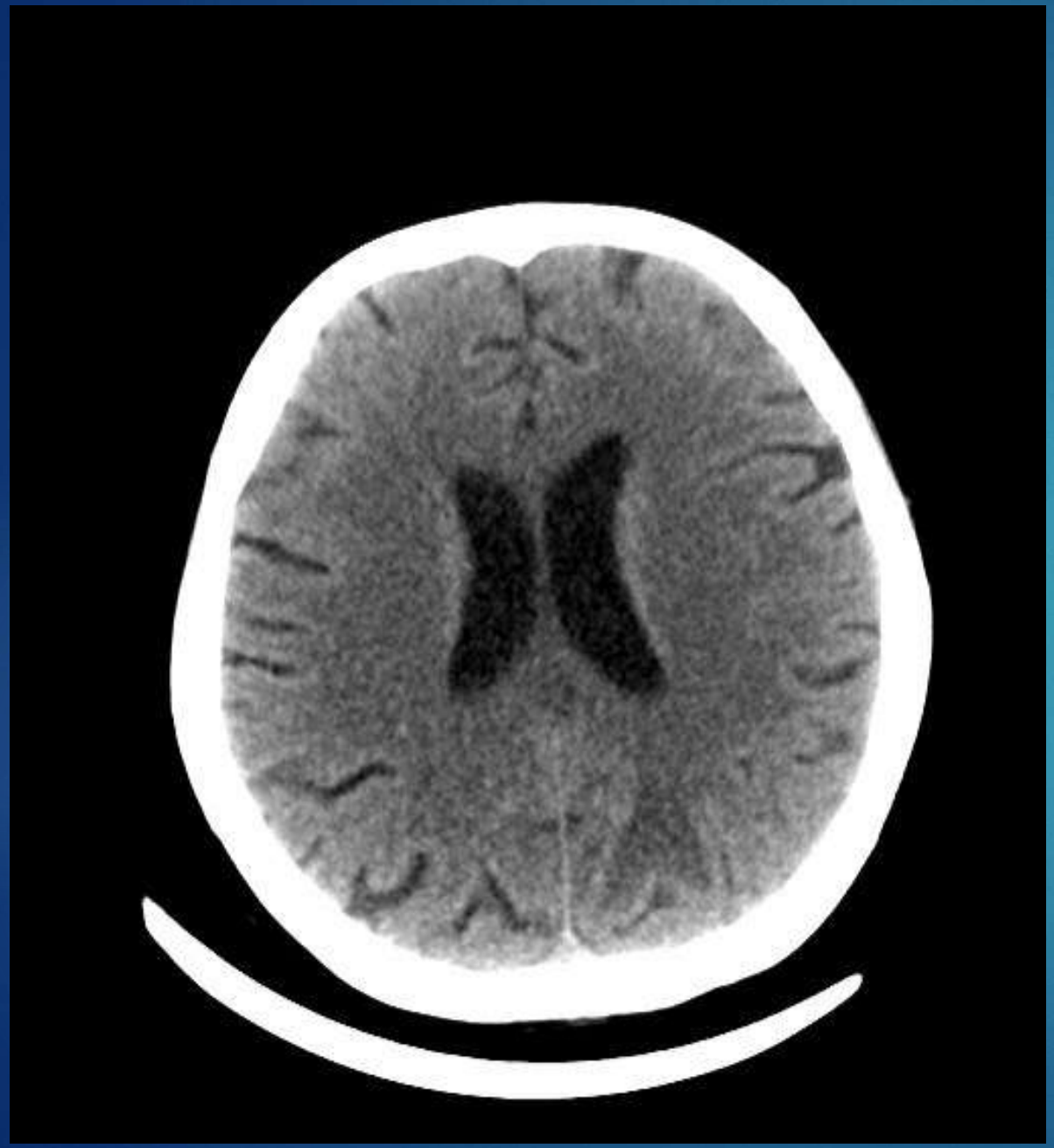


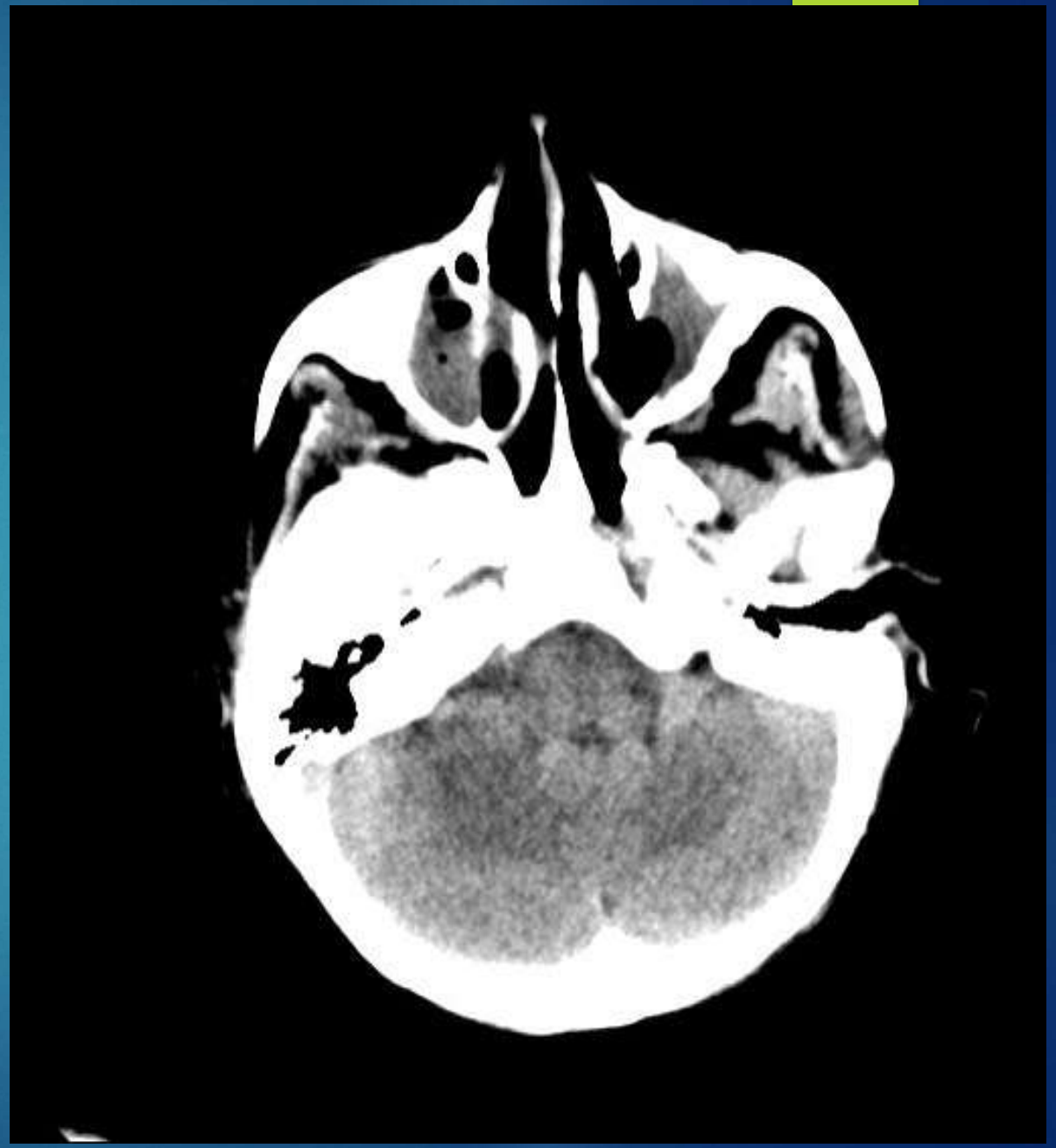
Central Nervous System Infections
Chapter 3
Uluhan Sili

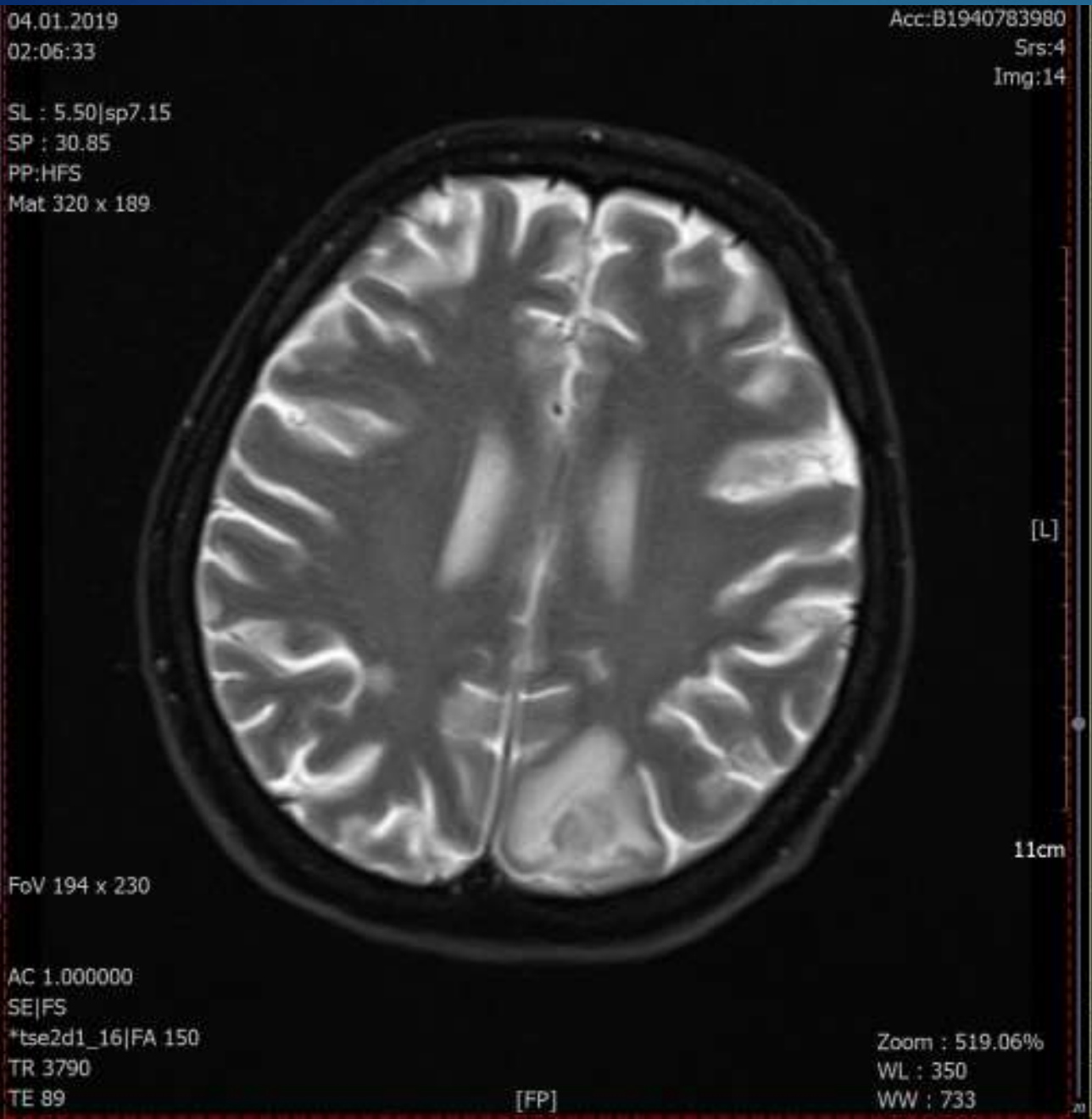
Endemic Infections in Turkey
Edited by Birgül Kaçmaz
Ankara Nobel Tıp Kitapevleri
2020

Olgu

- 49 yaş, kadın hasta
- 5 gündür süren sol ayakta güçsüzlük, ayakta duramama → Acil
- 1 aydır halsizlik, öksürük, balgam → toraks BT: PCP ile uyumlu
- GD orta, koopere-oriente, ense sertliği yok → kraniyal BT
- ateş 39.1°C
- pupiller anizokorik R>L, sağ göz tam pitotik
- serebeller testler bozuk



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WL : 350
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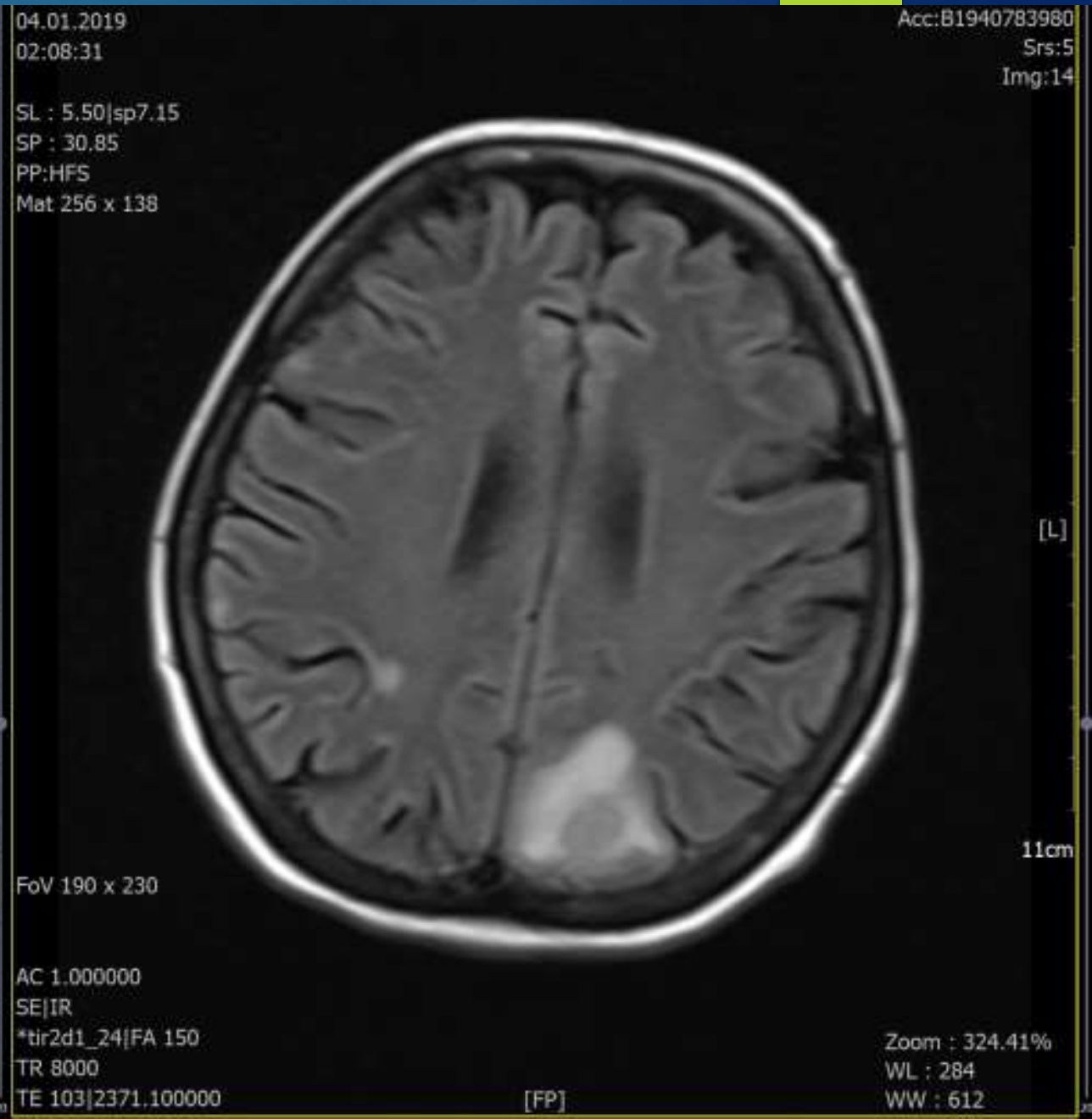
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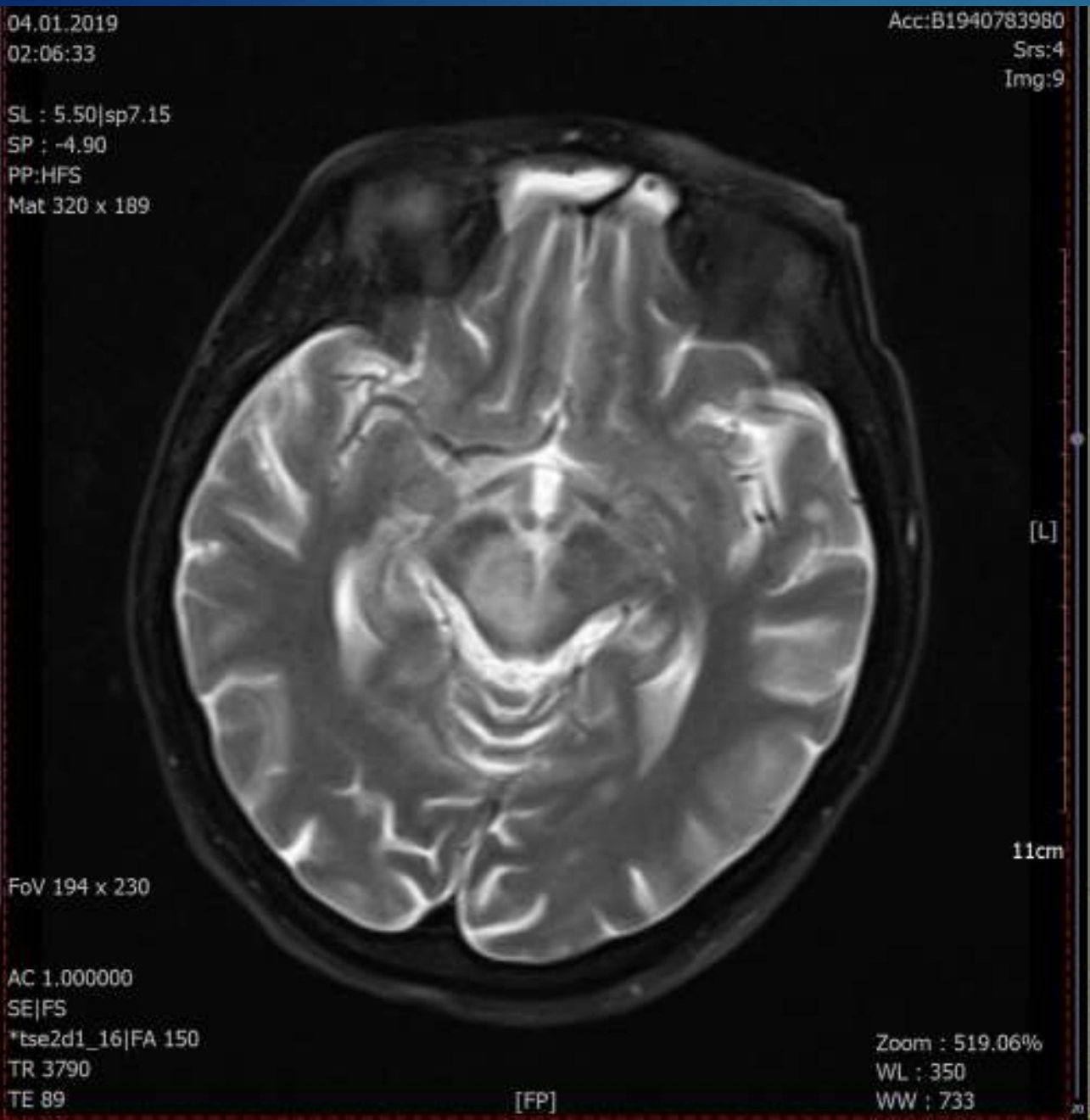
WW : 612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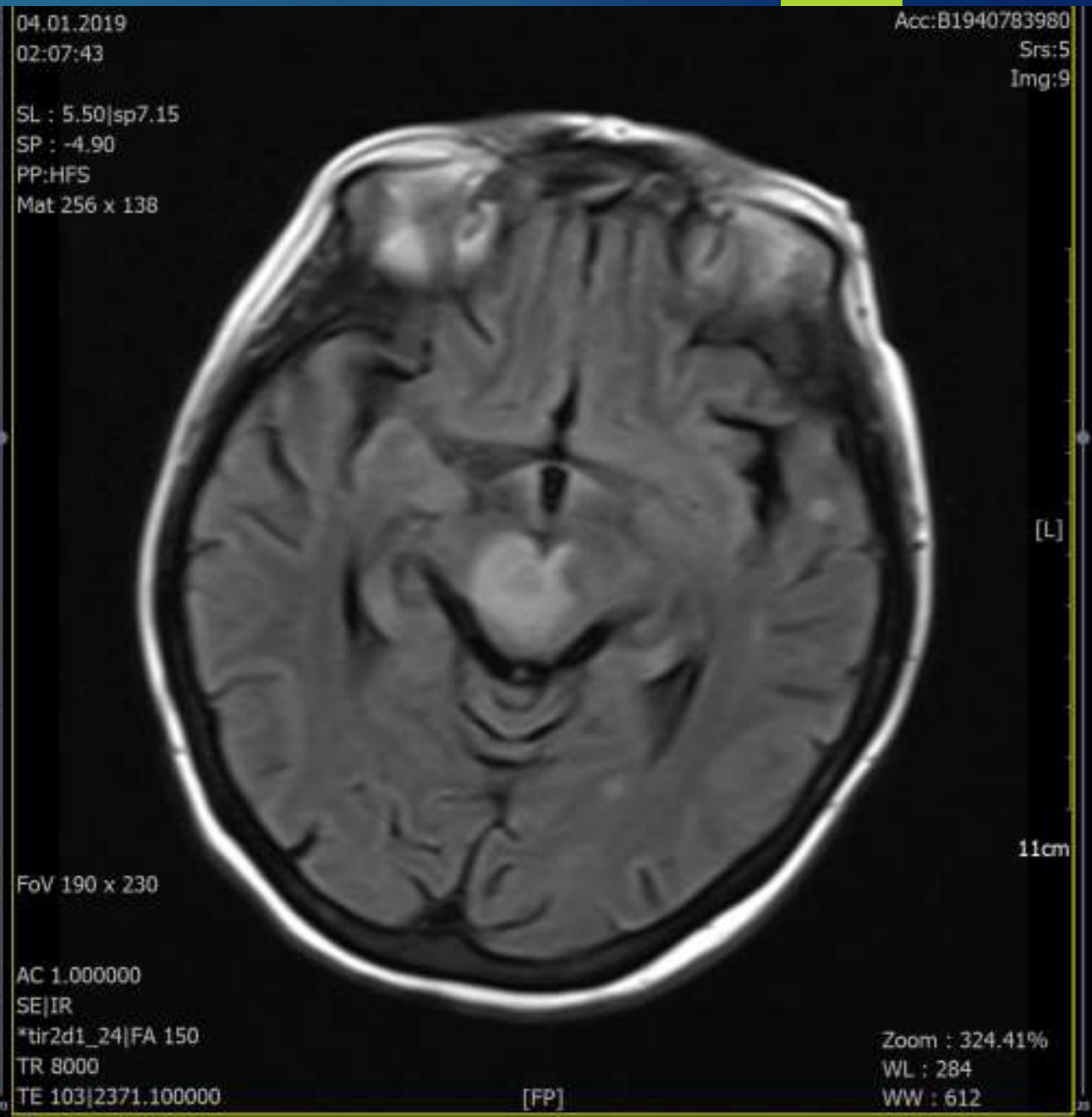
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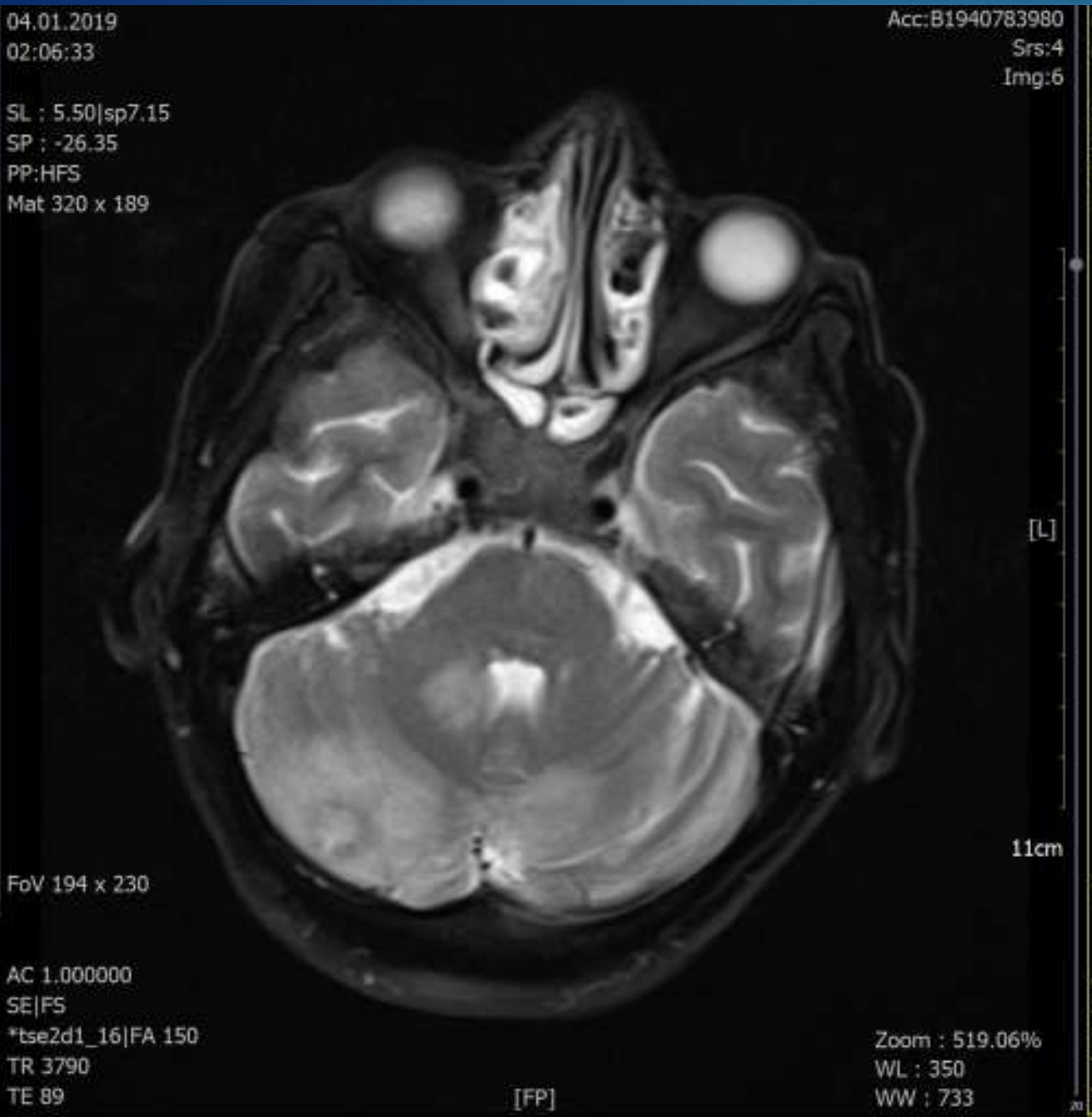
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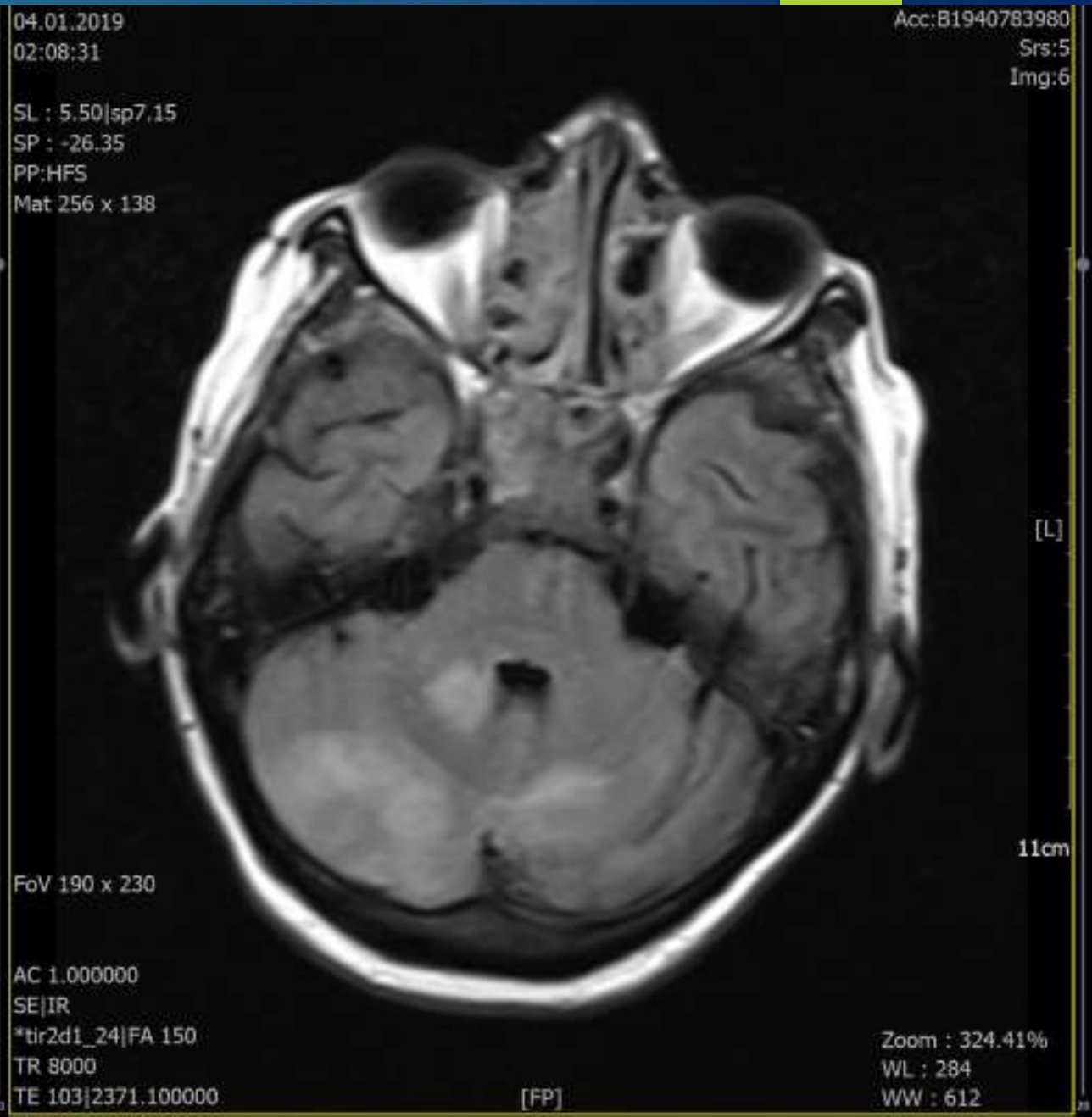
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WW : 612

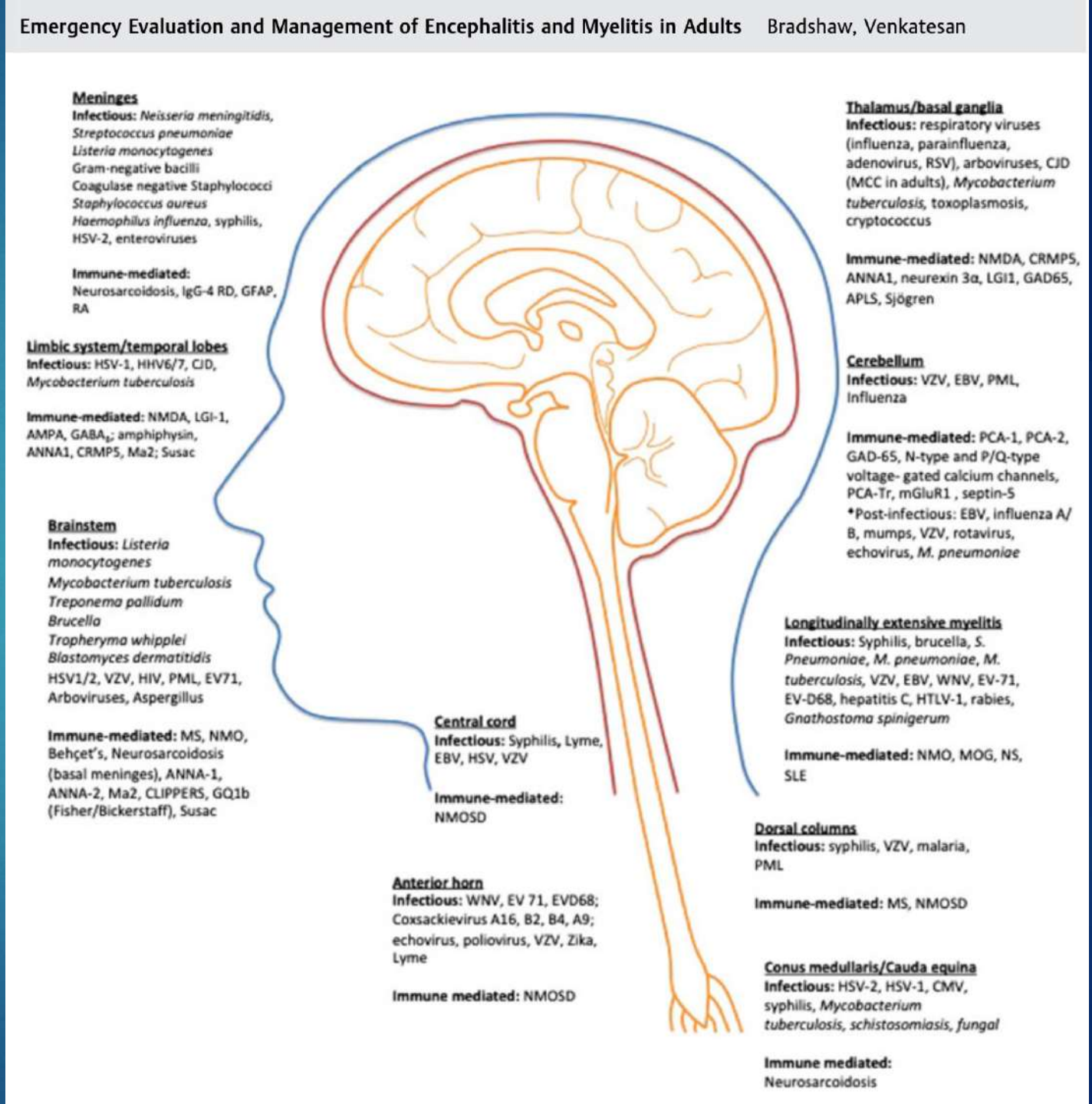
Olgu

- Anti-HIV (+), tekrarı (+)
- Lökosit 4900 hc/µL
  - lenfosit %6.1 (300 hc/µL)
  - CD4 %15 (30 hc/µL)
- anti-toksoplazma IgG (+)
  anti-CMV IgG (+)
  anti-VZV IgG (+)

- Empirik antimikrobiyal tedavi?

HIV/AIDS hastasında SSS infeksiyonu *Ayırıcı Tanısı*

- toksoplazma, kriptokok
- tüberküloz, nokardia, listeria
- stafilokok, streptokok
- HSV1/2, VZV, CMV, HHV-6, EBV, JC (PML), HIV
- lenfoma



BOS	
WBC	33 /µL
% PMNL	% 19.7
PMNL	6 /µL
% MNL	% 80.3
MNL	24 /µL
RBC	1305 /µL
Glukoz	41 mg/dL
Protein	95.3 mg/dL

Escherichia coli K1		Saptanamadı
Haemophilus influenzae		Saptanamadı
Listeria monocytogenes		Saptanamadı
Neisseria meningitidis		Saptanamadı
Streptococcus agalactiae		Saptanamadı
Streptococcus pneumoniae		Saptanamadı
Cytomegalovirus (CMV)	K	SAPTANDI

Film array menejit/ensefalit paneli CMV ya da HHV 6 için late
değerlendirilmesi önerilir

Enterovirus		Saptanamadı
Herpes simplex virus 1 (HSV-1)		Saptanamadı
Herpes simplex virus 2 (HSV-2)		Saptanamadı
Human herpesvirus 6 (HHV-6)		Saptanamadı
Human parechovirus		Saptanamadı
Varicella zoster virus (VZV)	K	SAPTANDI

Dr. şahika'ya bildirildi.

Cryptococcus neoformans/gattii		Saptanamadı

kopya/mL	BOS	Plazma
HIV-RNA	119,051	68,359
CMV-DNA	2,830,029	>3,000,000
VZV-DNA	<250	-
EBV-DNA	<250	-

- anti-retroviral tedavi ve gansiklovir başlandı
- 13. günde ponsa kanadı
  18. günde eksitus

Case Definitions, Diagnostic Algorithms, and Priorities in Encephalitis: Consensus Statement of the International Encephalitis Consortium

A. Venkatesan,[1] A. R. Tunkel,[2] K. C. Bloch,[3,4] A. S. Lauring,[5] J. Sejvar,[6] A. Bitnun,[7] J-P. Stahl,[8] A. Mailles,[9] M. Drebot,[10] C. E. Rupprecht,[11] J. Yoder,[12] J. R. Cope,[12] M. R. Wilson,[13,14] R. J. Whitley,[15,16,17,18] J. Sullivan,[19] J. Granerod,[20] C. Jones,[21,22] K. Eastwood,[23] K. N. Ward,[20,24] D. N. Durrheim,[25,26] M. V. Solbrig,[27] L. Guo-Dong,[28] and C. A. Glaser;[29] on behalf of the International Encephalitis Consortium

Clinical Infectious Diseases 2013;57(8):1114–28

Table 2. Diagnostic Algorithm for Initial Evaluation of Encephalitis in Adults[a]

ROUTINE STUDIES
CSF
Collect at least 20 cc fluid, if possible; freeze at least 5–10 cc fluid, if possible
Opening pressure, WBC count with differential, RBC count, protein, glucose
Gram stain and bacterial culture
HSV-1/2 PCR (if test available, consider HSV CSF IgG and IgM in addition)
VZV PCR (sensitivity may be low; if test available, consider VZV CSF IgG and IgM in ad
Enterovirus PCR
Cryptococcal antigen and/or India Ink staining
Oligoclonal bands and IgG index
VDRL
SERUM
Routine blood cultures
HIV serology (consider RNA)
Treponemal testing (RPR, specific treponemal test)
Hold acute serum and collect convalescent serum 10–14 d later for paired antibody testing
Immunocompromised—CMV PCR, HHV6/7 PCR, HIV PCR (CSF); *Toxoplasma gondii* serology and/or PCR; MTB testing[b]; fungal testing[c]; WNV testing[d]
Chest imaging (Chest x-ray and/or CT)
NEUROPHYSIOLOGY
EEG
OTHER TISSUES/FLUIDS
When clinical features of extra-CNS involvement are present, we recommend additional testing (eg, biopsy of skin lesions; bronchoalveolar lavage and/or endobronchial biopsy in those with pneumonia/pulmonary lesions; throat swab PCR/culture in those with upper respiratory illness; stool culture in those with diarrhea); also see below

HSV-1/2 PCR (if t
VZV PCR (sensiti
Enterovirus PCR
Cryptococcal ant

Journal of Infection (2012) 64, 347–373

ELSEVIER

BIAM British Infection Association

www.elsevierhealth.com/journals/jinf

Management of suspected viral encephalitis in adults — Association of British Neurologists and British Infection Association National Guidelines

T. Solomon [a,b,*,u], B.D. Michael [a,b,l,u], P.E. Smith [c,m], F. Sanderson [d,n], N.W.S. Davies [e,o], I.J. Hart [f,p], M. Holland [g,q], A. Easton [h,r], C. Buckley [i,s], R. Kneen [j,t], N.J. Beeching [k,p], On behalf of the National Encephalitis Guidelines Development and Stakeholder Groups

What virological investigations should be performed ?

Recommendations

- All patients with suspected encephalitis should have a CSF PCR test for HSV (1 and 2), VZV and enteroviruses, as this will identify 90% of cases due to known viral pathogens (B, II)
- Further testing should be directed towards specific pathogens as guided by the clinical features, such as occupation, travel history and animal or insect contact (B, III)

HIV (all patients)

-If positive:

- CSF PCR for EBV + CMV
- CSF TB staining + culture
- CSF + blood culture for *Listeria monocytogenes*
- CSF India ink staining +/or cryptococcal antigen for *Cryptococcus neoformans*
- CSF PCR + serology for *Toxoplasma gondii*
- CSF + serum antibody for syphilis

Consider:

- CSF PCR for HHV6 + 7
- CSF PCR for JC/BK virus

-CSF for Coccidioides + Histoplasma

Table 11 Microbiological investigations in patients with encephalitis, modified from (Solomon, Hart et al. 2007).[36]

CSF PCR	
1. All patients	HSV-1, HSV-2, VZV Enterovirus, parechovirus
2. If indicated	EBV/CMV (especially if immunocompromised) HHV 6,7 (especially if immunocompromised, or children) Adenovirus, influenza A &B, rotavirus (children) Measles, mumps Erythrovirus B19 Chlamydia
3. Special circumstances	Rabies, West Nile virus, tick-borne encephalitis virus (if appropriate exposure)

Guidelines on the management of infectious encephalitis in adults

Recommandations de prise en charge des encéphalites infectieuses de l'adulte

J.P. Stahl [a,*,1], P. Azouvi [b], F. Bruneel [c], T. De Broucker [d], X. Duval [e], B. Fantin [f], N. Girard [g], J.L. Herrmann [h], J. Honnorat [i], M. Lecuit [j,k], A. Mailles [l,1], L. Martinez-Almoyna [m], P. Morand [n], L. Piroth [o], P. Tattevin [p,1], The reviewing group[2]

Médecine et maladies infectieuses 47 (2017) 179–194

Grade A: herpes simplex virus (HSV), varicella zoster virus (VZV), and enterovirus PCR tests are imperative.

Grade A: HSV, VZV, and enterovirus PCR results must be available within 48 hours. The microbiologist must be contacted within the first 48 hours.

ESCMID guideline: diagnosis and treatment of acute bacterial meningitis

D. van de Beek[1], C. Cabellos[2], O. Dzupova[3], S. Esposito[4], M. Klein[5], A. T. Kloek[1], S. Leib[6], B. Mourvillier[7], C. Ostergaard[8], P. Pagliano[9], H. W. Pfister[5], R. C. Read[10], O. Resat Sipahi[11] and M. C. Brouwer[1], for the ESCMID Study Group for Infections of the Brain (ESGIB)

Clin Microbiol Infect 2016

TABLE 2.3. Causative organisms of adult bacterial meningitis

Country	Denmark [25]	Turkey [26]	United Kingdom [27]	Czech Republic [28]	Netherlands [4]	Total
Observation period	1998–2012	1994–2003	1997–2002	1997–2004	2006–2012	
Neisseria meningitidis	42	251	550	75	171	1089 (27%)
Streptococcus pneumoniae	92	457	525	82	1001	2157 (53%)
Haemophilus influenzae	3	2	48	3	56	112 (3%)
Listeria monocytogenes	5	6	48	21	74	154 (4%)
Other	30	68	124	35	291	548 (13%)
Total	172	784	1295	216	1593	4060

ESCMID guideline: diagnosis and treatment of acute bacterial meningitis

D. van de Beek[1], C. Cabellos[2], O. Dzupova[3], S. Esposito[4], M. Klein[5], A. T. Kloek[1], S. Leib[6], B. Mourvillier[7], C. Ostergaard[8], P. Pagliano[9], H. W. Pfister[5], R. C. Read[10], O. Resat Sipahi[11] and M. C. Brouwer[1], for the ESCMID Study Group for Infections of the Brain (ESGIB)

Diagnostic accuracy of laboratory techniques in bacterial meningitis

Level 2	In patients with a negative CSF culture and CSF Gram stain, PCR has additive value in the identification of the pathogen.

Recommendation

Grade A	In patients with suspected bacterial meningitis, it is strongly recommended to determine CSF leukocyte count, protein and glucose concentration, and to perform CSF culture and Gram stain.
Grade A	In patients with negative CSF cultures, the causative microorganisms can be identified by PCR and potentially by immunochromatographic antigen testing.
Grade A	In patients with suspected bacterial meningitis, it is strongly recommended to perform blood cultures before the first dose of antibiotics is administered.

- PCR'ın bakteriyel menenjit tanısı koymadaki **duyarlılığı**
  S. pneumonia 79-100%
  N. meningitidis 91-100%
  H. influenzae 67-100%
- 409 bakteriyel menenjit hastasının **%33**'ünün tanısı geleneksel yöntemlerle değil **sadece PCR** ile konulabilmiş (Parent du Chatelet I et al. 2005)
- 1099/1925 (**%57**) hastada invazif meningokokkal hastalık **sadece PCR** ile teyit edilebilmiş (Heinsbroek E et al. 2013)
- 46/188 (**%24**)pediatrik meningokok hastalığı **sadece PCR** ile teyit edilebilmiş. Kültür pozitif olanların **%5**'inde **PCR negatif!** (Munoz-Almagro C et al. 2009)
- PCR özellikle lumbar ponksiyon **öncesinde antibiyotik** alıp kültürleri negatifleşen hastalarda yararlı
- Dezavantajları:
  antimikrobiyal duyarlılık belirlenemiyor
  bakteriyel menenjit olgularının %5-26'sına *S. pneumonia, N. meningitidis, H. influenzae* **dışı bakteriler** neden olur (panelde var mı?)
- **PCR henüz BOS kültürünün yerine almaz ama faydalı bir ek test**
- European Monitoring Group on Meningococci (EMGM): **Meningokoktan** şüphelenildiğinde **PCR gerekli** (essential) bir test olarak kabul edilmektedir. (Fox AJ et al. 2007)

Meningitis Diagnostics Use Cases

World Health Organization

Nucleic acid tests. Nucleic acid tests (NAT) such as laboratory-based PCR are highly sensitive but require sophisticated laboratory infrastructure including biosafety cabinets to avoid contamination issues, as well as highly skilled laboratory technicians. Other assays such as LAMP are less susceptible to contamination but still require a modest laboratory environment and user training. Multiplex PCR panels have been developed for meningitis pathogens and syndromic diseases using PCR kits for (3 species per tube), and array-based platforms (14-20 species per array); these platforms require the highest level of skill and laboratory infrastructure.[10,11] At the other end of the spectrum, point-of-care (POC) nucleic acid tests are fully automated and can be run in a near-patient laboratory by clinic staff after moderate training, though POC meningitis assays are currently available only for tuberculosis meningitis.[12,13]

Since many of the symptoms of meningitis are similar to other endemic diseases, it is critical to differentiate the source of infection from other syndromic pathogens in order to determine the appropriate treatment regimen, including as switch treatment or terminate inappropriate treatment. Given the complexity of the assay, it would likely be implemented at hospitals with higher infrastructure. Ideally the test would differentiate pathogens such as *N.meningitidis, S.pneumoniae, H.influenzae* type b, Salmonella, Listeria, Group B streptococci, echovirus, coxsackievirus, varicella zoster virus, enterovirus, herpes simplex, Cryptococcus from CSF or venous whole blood. The assay needs to be highly specific and sensitive to influence case management (stopping /changing antibiotics).[1] The test happens at hospital level on samples from in-patients or on samples referred from a health facility that is geographically close.

Use Case 1: Epidemic/outbreak setting (Africa) – Identification of Nm serogroup at peripheral level (health center or district hospital) for appropriate vaccine response

Use Case 2: Epidemic and endemic settings (worldwide) – Identification of bacterial meningitis/septicaemia at peripheral level (health clinic or hospital) to initiate antibiotic treatment (yes/no) for case management

Use Case 3: Epidemic and endemic settings: Syndromic meningitis panel (minimum 10 pathogens) at hospital level (district/regional hospital) for case management: stopping or changing antibiotics

Pathogen:	Meningitis (including bacteria, viral, fungal infection)
UC 1A:	Use Case 1 - Differential detection of Nm serogroups; A (LFA/RDT)
UC 1B:	Use Case 1 - Differential detection of Nm serogroups; B (LFA/RDT, LAT, EIA)
UC 2A:	Use Case 2 -Identification of bacterial meningitis (vs. viral or non-meningitis disease); A (LFA/RDT)
UC 2B:	Use Case 2 - Identification of bacterial meningitis (vs. viral or non-meningitis disease); B (LFA/RDT, LAT, EIA, POC-NAT)
UC 3A:	Use Case 3 - Syndromic panel for detection and differentation of meningitis; A (LFA/RDT, LAT, POC-NAT)
UC 3B:	Use Case 3 - Syndromic panel for detection and differentation of meningitis; A (LFA/RDT, LAT, EIA, POC-NAT, lab-NAT)

NAT kit multiplex							**Fast-track Diagnostics**	**FTD Neonatal Meningitis**	CE	http://www.fast-trackdiagnostics.com/human-line/products/ftd-neonatal-meningitis/	Cerebrospinal fluid (CSF) and EDTA or citrated blood sample	Quantitative detection of **bacteria**: Streptococcus Group B, Listeria monocytogenes, Escherichia coli
NAT kit multiplex							**Fast-track Diagnostics**	**FTD Viral Meningitis**	CE	http://www.fast-trackdiagnostics.com/human-line/products/ftd-viral-meningitis/	Cerebrospinal fluid (CSF) and blood (without heparin)	Quantitative detection of **viruses**: herpes simplex virus 1 and 2, varicella zoster virus, enterovirus, mumps virus, human parechovirus
NAT kit multiplex							**Fast-track Diagnostics**	**FTD Neuro 9**	CE	http://www.fast-trackdiagnostics.com/human-line/products/ftd-neuro-9/	Cerebrospinal fluid (CSF) and blood (without heparin)	4 tubes: Quantitative detection of **viruses**: human cytomegalovirus, Epstein-Barr virus, human adenovirus, herpes simplex virus 1 and 2, varicella zoster virus, enterovirus, human parechovirus, human herpesvirus 6 and 7, human parvovirus B19
NAT kit multiplex							**Fast-track Diagnostics**	**FTD EPA**	CE	http://www.fast-trackdiagnostics.com/human-line/products/ftd-epa/	CSF, blood (without heparin), throat swabs, sputum and stool	Quantitative detection of **viruses** enterovirus, human parechovirus, human adenovirus
NAT kit multiplex				?			**Sacace Biotechnologies** (Italy)	**NHS Meningitidis**	CE	https://sacace.com/neurological-infections.htm#s2	n/a	detection of **bacteria:** Neisseria meningitidis, Haemophilus influenzae and Streptococcus pneumoniae
NAT kit multiplex						X	**Seegene** (KOR)	**Allplex™ Meningitis Panel 1, 2, 3**	CE	http://www.seegene.com/neo/en/products/meningitis/allplex_meningitis_fp.php	cerebrospinal fluid (CSF)	Panel 1 (**Viruses**): Herpes simplex virus type 1, Herpes simplex virus type 2, Varicella-zoster virus, Epstein-barr virus, Cytomegalovirus, Human herpesvirus 6, Human herpesvirus 7; Panel 2 (**Viruses**): Parvovirus B19, Mumps virus, Parechovirus, Adenovirus, Enterovirus; Panel 3 (**Bacteria**): Haemophilus influenza, Streptococcus pneumoniae, Listeria monocytogenes, Neisseria meningitides, Group B Streptococcus, E.coli K1
NAT kit multiplex						X	**Seegene** (KOR)	**Seeplex® Meningitis ACE Detection Panels 1, 2, 3**	CE	http://www.seegene.com/neo/en/products/meningitis/seeplex_MENINGITIS.php	cerebrospinal fluid (CSF)	Panel 1 (**Viruses**): HSV 1, HSV 2, VZV (HHV3), EBV (HHV4), CMV (HHV5)(CE0086), HHV6; Panel 2 (**Viruses**): Enteroviruses; Panel 3 (**Bacteria**): Streptococcus pneumoniae, Neisseria meningitides, Haemophilus influenza, Listeria monocytogenes, Group B Streptococcus, (Streptococcus agalactiae)
NAT kit multiplex						X	**DiagCor** (KOR)	**GENOFLOW R14004**		http://www.medicalexpo.com/prod/diagcor-bioscience-incorporation-limited/product-83412-778647.html	CSF	detects 12 common **bacterial** pathogens for meningitis
NAT kit multiplex						X	**Pathofinder** (NL)	**MeningoFinder® 2SMART**	CE	http://www.pathofinder.com/products/twosmartfinder/meningofinder-2smart	cerebrospinal fluid (CSF)	**Viruses:** HSV 1/2, VZV, EBV, CMV, Human Herpesvirus 6/7/8, Human enterovirus, Parechovirus, Mumps, Measles; **Bacteria:** Listeria monocytogenes, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus agalactiae, Neisseria meningitides, Borrrelia burgoferi/miyamatoi, Echerichia coli K1
NAT kit multiplex platform						X	**BioFire Diagnostics** (USA) **/bioMerieux**	**Meningitis Encephalitis Panel (14 targets)**	CE	http://www.biofiredx.com/products/the-filmarray-panels/	CSF	**BACTERIA:** E.coli K1, H.influenza, L.monocytogenes, N.meningitides, Streptococcus agalactiae, Streptococcus pneumoniae; **VIRUSES:** Cytomegalovirus (CMV), Enterovirus, Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), Human herpesvirus 6 (HHV-6), Human parechovirus, Varicella zoster virus (VZV)

THE BIOFIRE ME PANEL MENU

Overall 94.2% Sensitivity and 99.8% Specificity
Sample Type: Cerebrospinal Fluid (CSF)

The BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel

BACTERIA:

- *Escherichia coli* K1
- *Haemophilus influenzae*
- *Listeria monocytogenes*
- *Neisseria meningitidis*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*

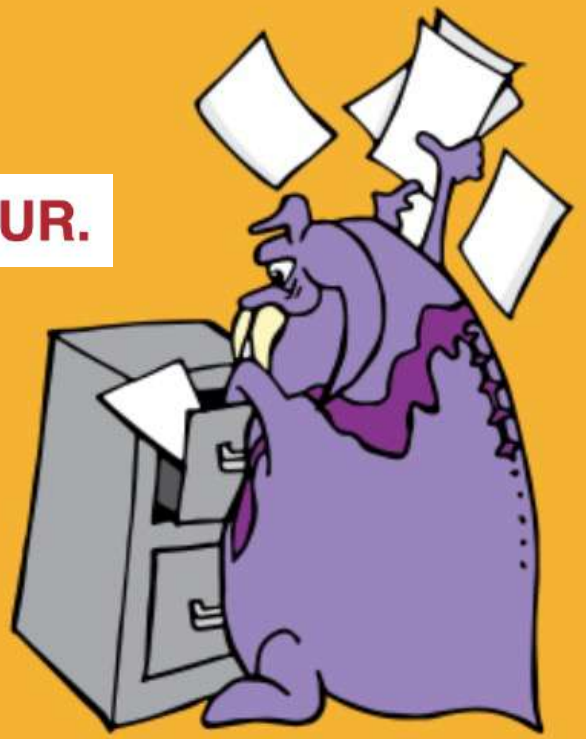
VIRUSES:

- Cytomegalovirus (CMV)
- Enterovirus (EV)
- Herpes simplex virus 1 (HSV-1)
- Herpes simplex virus 2 (HSV-2)
- Human herpesvirus 6 (HHV-6)
- Human parechovirus (HPeV)
- Varicella zoster virus (VZV)

YEAST:

- *Cryptococcus neoformans/gattii*

1 TEST. 14 PATHOGENS. 1 HOUR.



BIOMÉRIEUX

Bio-Speedy®

MENENJİT/ENSEFALİT
RT-qPCR MX-17S PANELİ

bioeksen

VİRAL ETKENLER

- Herpes Simpleks Virüsü 1
- Herpes Simpleks Virüsü 2
- İnsan Herpesvirüsü 6
- Sitomegalovirüs (CMV)
- Enterovirüs
- Parekovirüs
- Varisella Zoster Virüs
- İnsan Herpesvirüsü 7
- İnsan Herpesvirüsü 8

BAKTERİYEL ETKENLER

- *Listeria monocytogenes*
- *Neisseria meningitidis*
- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Escherichia coli K1*
- *Streptococcus agalactiae*

FUNGAL ETKENLER

- Cryptococcus gattii/neoformans

Geleneksel Yöntemlere Kıyasla	Bioeksen	Rakip A	Rakip B
Duyarlılık	**100**	94,2	96
Özgüllük	**98,24**	99,8	98,3

LOD: 10-100 kopya/ml
Duyarlılık > %100
Özgüllük > %98,24

Diagnostic test accuracy of the BioFire® FilmArray® meningitis/encephalitis panel: a systematic review and meta-analysis

G.S. Tansarli [1, 2], K.C. Chapin [1, 2, *]

Clinical Microbiology and Infection 26 (2020)

- FilmArray meningitis/encephalitis (ME) panel
- SSS infeksiyonlarına sık neden olan patojenleri saptar
- yanlış pozitif ve yanlış negatif olgular bildirildi
- **tanısal test kesinliği**
  diagnostic test accuracy
- ME paneli ile referans standart yöntemi karşılaştıran çalışmaların meta-analizi
  - toplum kaynaklı ME olguları
- 13 çalışma (3764 hasta)
- duyarlılık= 90% (95% CI 86-93%)
- özgüllük= 97% (95% CI 94-99%)
- yanlış pozitif= 11.4% (4% kararlaştırma sonrası)
  - en sık yanlış pozitif *S. pneumoniae, S. agalactiae*
- yanlış negatif= 2.2% (1.5% kararlaştırma sonrası)
  - en sık yanlış negatif HSV 1/2, enterovirus, *Cryptococcus neoformans/gattii*
  - yanlış negatif *C. neoformans/gattii* hastaları tedavi almış veya almakta olan antijen pozitif hastalar
- BioFire ME paneli **yüksek tanısal kesinliğe** sahip

Dr. Tansarli and Dr. Chapin report grants from BioFire Diagnostics, LLC, for work outside of this work.

Impact of the FilmArray meningitis/encephalitis panel on antimicrobial duration among patients with suspected central nervous system infection

https://doi.org/10.1016/j.diagmicrobio.2021.115394

Kellie J. Goodlet[a,*], Elaine Tan[a], Lindsey Knutson[a], Michael D. Nailor[b]

Diagnostic Microbiology and Infectious Disease 100 (2021) 115394

Summary of controlled studies evaluating changes in antimicrobial duration with implementation of the FilmArray meningitis encephalitis multiplex PCR panel for patients with suspected central nervous system infection.

Author (Year)	Patient population	FAME group	Control group	Organism positivity	Turnaround time (hours)	Length of stay (days)	Antibiotic duration (days)	Acyclovir duration (days[b])
Broadhurst (2020)	Pediatric and adult patients with suspected HSV encephalitis (U.S.)	38 patients (-) for HSV; 2/2017-3/2019	39 patients (-) for HSV; 5/2016-12/2016	0% HSV (by design)	NR	NR	NR	1.3 vs 2.5; $P = 0.03$
Dack (2019)	Adult patients with suspected meningitis and CSF pleocytosis (U.S.)	47 patients; 8/2016-8/2017	50 patients; 8/2015-8/2016	42.6% (18/20 viral, HSV unknown) vs NR	NR	4 vs 4; $P = 0.935$	3 vs 3 (antibiotic vs acyclovir not differentiated); $P = 0.572$	
Evans (2019)	Pediatric and adult patients with suspected meningoencephalitis and receipt of ≥ 1 acyclovir dose (U.S.)	76 patients; 4/2017-12/2017	132 patients with ≥ 1 viral PCR result; 4/2016-12/2016	25% (14/19 viral, 3 HSV) vs 10.6% (9/14 viral, 1 HSV)	6.2 vs 37.9; $P < 0.01$	5.2 vs 6.1; $P = 0.59$	1.9 vs 1.6; $P = 0.28$	1.3 vs 1.7; $P < 0.01$
Hagen (2020)	Pediatric patients with suspected CNS infection and antibiotics or acyclovir prescribed (Germany)	46 patients; 6/2016-2/2017	46 matched patients; 1/2012-5/2016	30.4% vs 10.9% (all viral, 0 HSV)	NR	5 vs 5; $P = 0.384$	4 vs 6; $P = 0.023$	1 vs 3; $P < 0.001$
Messacar (2020)	Pediatric patients (age >60 d) with suspected CNS infection (U.S.)	408 patients; 1/2017-12/2017	4888 patients; 1/2007-1/2017	4.2% (11/17 viral, 0 HSV) vs 4.7% (161/231 viral, 8 HSV)	NR	NR	NR	3 vs 5 doses; $P = 0.05$
Mina (2019)	Pediatric (age >3 mo) and adult patients with CSF pleocytosis >100 cells/uL and (-) CSF or blood cultures (Israel)	8 patients (+) for bacteria per FAME; 6/2016-6/2018	23 patients; 1/2010-5/2016	100% (8/8 bacterial) vs 0% (by design)	NR	10 vs 17; $P = 0.065$	9.5 vs 15.2 (antibiotic vs acyclovir not differentiated); $P = 0.007$	
Moffa (2020)	Adult patients with suspected CNS infection (U.S.)	79 patients; 10/2017-9/2018	81 patients; 10/2016-9/2017	11.4% vs 9.9% (all viral, 4/9 vs 3/8 HSV)	4.1 vs 85-124[a]; $P < 0.001$	4.4 vs 6.6; $P = 0.02$	5 vs 7; $P < 0.001$	1 vs 3; $P < 0.001$
Nabower (2019)	Pediatric patients with CSF testing ≤ 48 h from admission (U.S.)	223 patients; 7/2016-7/2017	348 patients; 7/2015-7/2017	24.7% (38/55 viral, 1 HSV) vs 14.7% (46/51 viral, 2 HSV)	Bacterial: 2.2 vs 34; HSV: 2.2 vs 23.2	2.5 vs 2.8; $P = 0.049$	1.8 vs 1.9; $P = 0.14$	1 vs 3 doses; $P < 0.001$
Posnakoglou (2020)	Pediatric patients with CSF pleocytosis (Greece)	71 patients; 4/2018-4/2019	71 patients; 4/2018-4/2019 (randomized, prospective)	52.1% (27/37 viral, 0 HSV) vs 22.5% (11/16 viral, 0 HSV); $P < 0.001$	NR	5 vs 8; $P < 0.001$	4 vs 7; $P < 0.001$	3 vs 5; $P < 0.001$
Walker (2020)	Adult patients with CSF pleocytosis and (+)FAME result (U.S.)	91 patients; 6/2016-9/2016	72 patients; 6/2015-9/2015	NR	2.4 vs 132; $P < 0.001$	8 vs 8; $P = 0.623$	3 vs 5; $P = 0.156$	2 vs 3; $P = 0.396$

CNS = central nervous system; CSF = cerebrospinal fluid; FAME = FilmArray meningitis encephalitis multiplex PCR panel; HSV = herpes simplex virus 1 or 2; NR = not reported.

[a] Dependent on viral pathogen (HSV = median 85 hours).

[b] Unless otherwise specified.

Author (Year)	Turnaround time (hours)	Length of stay (days)	Antibiotic duration (days)	Acyclovir duration (days[b])
Broadhurst (2020)	NR	NR	NR	1.3 vs 2.5; $P = 0.03$
Dack (2019)	NR	4 vs 4; $P = 0.935$	3 vs 3 (antibiotic vs acyclovir not differentiated); $P = 0.572$	
Evans (2019)	6.2 vs 37.9; $P < 0.01$	5.2 vs 6.1; $P = 0.59$	1.9 vs 1.6; $P = 0.28$	1.3 vs 1.7; $P < 0.01$
Hagen (2020)	NR	5 vs 5; $P = 0.384$	4 vs 6; $P = 0.023$	1 vs 3; $P < 0.001$
Messacar (2020)	NR	NR	NR	3 vs 5 doses; $P = 0.05$
Mina (2019)	NR	10 vs 17; $P = 0.065$	9.5 vs 15.2 (antibiotic vs acyclovir not differentiated); $P = 0.007$	
Moffa (2020)	4.1 vs 85-124[a]; $P < 0.001$	4.4 vs 6.6; $P = 0.02$	5 vs 7; $P < 0.001$	1 vs 3; $P < 0.001$
Nabower (2019)	Bacterial: 2.2 vs 34; HSV: 2.2 vs 23.2	2.5 vs 2.8; $P = 0.049$	1.8 vs 1.9; $P = 0.14$	1 vs 3 doses; $P < 0.001$
Posnakoglou (2020)	NR	5 vs 8; $P < 0.001$	4 vs 7; $P < 0.001$	3 vs 5; $P < 0.001$
Walker (2020)	2.4 vs 132; $P < 0.001$	8 vs 8; $P = 0.623$	3 vs 5; $P = 0.156$	2 vs 3; $P = 0.396$

- "BioFire FilmArray Meningitis Encephalitis" paneli BOS kültürü ile birlikte kullanıldığında organizma saptama duyarlılığını %90'lara çıkarıyor
  - gerçek hayatta gereksiz antimikrobiyal kullanımını azaltabillir mi?
- Sonuç bildirimi çok daha hızlı
  - 2.2 – 6.2 saate 24 veya daha uzun
- Çalışmaların yarısı antibiyotik süresinde anlamlı düşüş göstermiş
- 8/10 çalışma asiklovir süresinde düşük düzey ama anlamlı kısalma göstermiş
- Pozitif sonuç antimikrobiyal değiştirmede negatif sonuca göre daha etkili
  - negatif çıksa da antibiyotiğe devam ediliyor ← müdahale edilmeli

Impact of a Multiplex Polymerase Chain Reaction Panel on Duration of Empiric Antibiotic Therapy in Suspected Bacterial Meningitis

Justin J. Choi,[1] Lars F. Westblade,[2,3] Lee S. Gottesdiener,[1] Kyle Liang,[1] Han A. Li,[1] Graham T. Wehmeyer,[1] Marshall J. Glesby,[3] and Matthew S. Simon[3]

[1]Division of General Internal Medicine, Department of Medicine, Weill Cornell Medicine, New York, New York, USA, [2]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, New York, USA, [3]Division of Infectious Diseases, Department of Medicine, Weill Cornell Medicine, New York, New York, USA

Open Forum Infectious Diseases®2021

https://doi.org/10.1093/ofid/ofab467

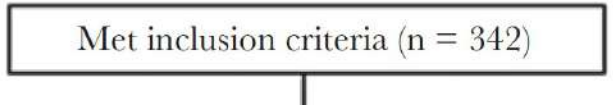


Table 2. Antimicrobial Use and Hospitalization Outcomes

Clinical Outcome	Pre-multiplex PCR ME Panel (n = 137)	Post-multiplex PCR ME Panel (n = 69)	*P* Value
Duration of empiric antimicrobial therapy, hours, median (IQR)	34.7 (8.5–61.7)	12.3 (3.3–40.0)	.01
Duration of total antimicrobial therapy in hospital, hours, median (IQR)	39.6 (10.3–86.0)	14.3 (4.3–64.9)	.02
Time to targeted therapy, hours, median (IQR)	59.3 (36.5–74.6)[a]	7.02 (0.9–12.4)[b]	<.001
Patients hospitalized, n (%)	126 (92.0)	63 (91.3)	.87
Hospital length of stay, days, median (IQR)	4 (2–7)	3 (1–5)	.03
In-hospital mortality, n (%)	3 (2.2)	2 (2.9)	1.00

Diagnostic challenges of central nervous system infection: extensive multiplex panels versus stepwise guided approach

P. Vetter [1, 2, *], M. Schibler [1, 2], J.L. Herrmann [3, 4], D. Boutolleau [5, 6]

Clinical Microbiology and Infection 26 (2020)

Main advantages and limitations of molecular multiplex assays such as the FilmArray® ME panel

Advantages	Limitations
Easy to use	Not fully comprehensive: does not exclude an infection
Sealed format	Specific epidemiology not considered
Low turnaround time	Not adapted for nosocomial infections or immunocompromised patients
Multiple targets tested simultaneously	Too extensive for most clinical situations (i.e. meningoencephalitis in non-traveller immunocompetent individuals)
Potential decreased length of stay	Lower sensitivity than conventional singleplex assays for most targets
Decreased health costs by rapid herpes simplex virus and enterovirus results	Lack of clinical validation for most targets
Decreased antimicrobial use (mainly acyclovir)	Need for confirmation test for some targets

Diagnostic challenges of central nervous system infection: extensive multiplex panels versus stepwise guided approach

P. Vetter [1, 2, *], M. Schibler [1, 2], J.L. Herrmann [3, 4], D. Boutolleau [5, 6]

Clinical Microbiology and Infection 26 (2020)

Suspec
CNS infe

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Suggested algorithm

If patient immunocompromised, call ID specialist

HIV Serology
CSF cell count, glucose and protein level, aspect of the CSF

Purulent CSF
Elevated number of white blood cells
High neutrophil count
Low glucose
High protein

Clear CSF
Elevated number of white blood cells
High lymphocytes count
Normal to low glucose
Normal to high protein

Gram stain
Bacterial culture

Small bacterial panel:
S. pneumoniae, N. meningitidis

Small viral panel:
HSV-1, HSV-2, VSV, EV

Gram stain

⊖ Call ID specialist and discuss additional testing for bacteria depending on exposure/risk factors

⊕ Adapt clinical management accordingly

⊕ Bacterial/Mycobacterial culture
Syphilis serology
Borrelia serology
TBE PCR/serology

⊖ Discuss additional testing with ID specialist depending on exposure/risk factors/imaging/EEG
GenXpert TB, IGRA, CrAg, CMV, EBV, HHV-6, JCV, Rabies, LCMV, MeV,
Arboviruses PCR/serology (WNV, Toscana virus…), intrathecal antibody synthesis (HSV, VZV, MeV…) …

Toplum Kökenli Santral Sinir Sistemi Enfeksiyonlarında Bakteriyel ve Viral Etiyolojinin Moleküler Yöntemlerle Değerlendirilmesi

Mikrobiyol Bul 2017; 51(3): 277-285

doi: 10.5578/mb.57358

Hasip KAHRAMAN[1], Alper TÜNGER[2], Şebnem ŞENOL[3], Hörü GAZİ[4], Meltem AVCI[5], Bahar ÖRMEN[6], Nesrin TÜRKER[6], Sabri ATALAY[7], Şükran KÖSE[7], Sercan ULUSOY[8], Meltem IŞIKGÖZ TAŞBAKAN[8], Oğuz Reşat SİPAHİ[8], Tansu YAMAZHAN[8], Zeynep GÜLAY[9], Sema ALP ÇAVUŞ[10], Hüsnü PULLUKÇU[8]

Tablo I. PCR Yöntemiyle İzole Edilen Etkenlerin Dağılımı

Bakteriyel menenjit olguları	
İzole edilen etkenler	**Sayı**
Streptococcus pneumoniae	14
Neisseria meningitidis	2
Haemophilus influenzae	1
Listeria monocytogenes	1
S.pneumoniae + N.meningitidis	1
S.pneumoniae + enterovirüs	2
S.pneumoniae + EBV	2
N.meningitidis + enterovirüs	2
S.pneumoniae + HHV-6	1
Grup B streptokok + enterovirüs	1
Toplam	**27**
Viral menenjit/ensefalit olguları	
İzole edilen etkenler	**Sayı**
Enterovirüs	7
HSV-1	2
HSV-2	1
VZV	1
Enterovirüs + HSV-1	1
Enterovirüs + EBV	1
HSV-1 + EBV	1
Toplam	**14**

HSV: Herpes simpleks virüs; EBV: Ebstein Barr virüs.

- çok merkezli, prospektif kohort
- toplum kökenli SSS infeksiyonları
- bakteriyel ve viral etkenlerin bakteriyel kültür ve multipleks PCR yöntemiyle araştırılması
- Nisan 2012 – Şubat 2014
  - SSS infeksiyonu bulguları ile başvuran 92 hasta
- Seeplex meningitis-B, -V1 ve -V2 panelleri kullanılmış
- 79 hasta: 41 hastada panelle etken gösterilmiş
  - bakteriyel menenjit düşünülen 46 hastanın:
    konvansiyonel kültürle 10 (%21.7)
    9 pnömokok, 1 *H. influenzae*
    panel ile 27 (%58.6) hastada etken gösterilmiş
  - viral menenjit/ ensefalit düşünülen 33 hastanın:
    panel ile 13'ünde etken saptanmış
- Panel ile SSS infeksiyonlarında etkeni saptayabilme oranları artmış
  - çoklu etken saptamanın klinik anlamı?

Sağlık Uygulama Tebliği'nde 8 Şubat 2022'de yayınlanan değişiklik tebliği ile Tıbbi Mikrobiyoloji Laboratuvarı Moleküler Mikrobiyoloji biriminde halen çalışılan bazı testlerin SUT kodları iptal edilmiştir. Bu nedenle bu SUT kodlarını içeren testler faturalandırılamayacaktır. Bu kalemlerin çalışılamaması, özellikle üçüncü basamak sağlık hizmeti sunan kurumlarda, tanı ve tedavi süreçlerinde hastaların aleyhine işleyecektir.

Konu olan testler;

- Solunum Sistemi Patojenleri Real Time PCR testi
- Menenjit-Ensefalit Etkenleri Real Time PCR testi
- Gastrointestinal Sistem Patojenleri Real Time PCR testi gibi birden fazla parametre içeren sendromik testler ile,

- Pneumocystis jirovecii Real Time PCR testi
- BK Virus Real Time PCR testi
- Adenovirus Real Time PCR testi
- Enterovirus Real Time PCR testi gibi spesifik viral etkenlerin moleküler tanısına yönelik testler
- Antiviral direnç tayini
- Genotipik analiz gibi DNA dizi analizi vb. moleküler mikrobiyolojik testlerdir

Hem devam etmekte olan ihale süreçlerine ait geri ödemelerin yapılabilmesi, hem de ileriye dönük sorun yaşanmaması için Mikrobiyoloji SUT Kodlarının hızla düzenlenmesi, bu çalışma tamamlanıncaya kadar, kaldırılan PCR multipleks, Real-time PCR 11 ve üzeri reaksiyon ve RT-PCR kodlarının ACİLEN SUT 2B listesine tekrar eklenmesi gerekmektedir.

Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis

N Engl J Med 2019;380:2327-40.
DOI: 10.1056/NEJMoa1803396

BACKGROUND

Metagenomic next-generation sequencing (NGS) of cerebrospinal fluid (CSF) has the potential to identify a broad range of pathogens in a single test.

METHODS

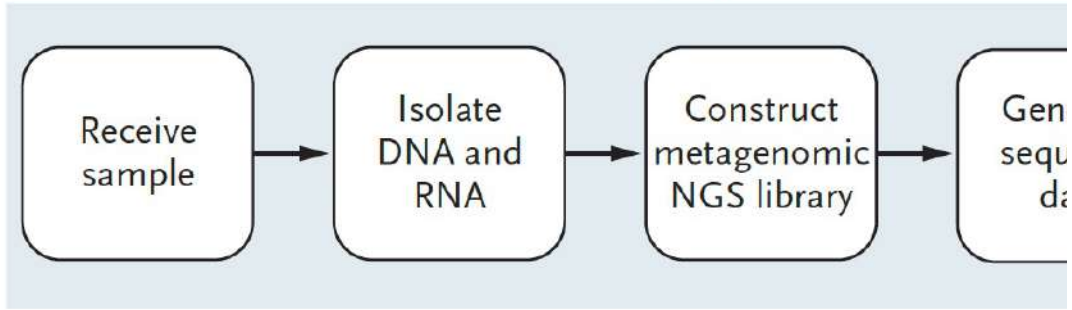
In a 1-year, multicenter, prospective study, we investigated the usefulness of metagenomic NGS of CSF for the diagnosis of infectious meningitis and encephalitis in hospitalized patients. All positive tests for pathogens on metagenomic NGS were confirmed by orthogonal laboratory testing. Physician feedback was elicited

owing to low titers of pathogens in CSF. A total of 8 of 13 diagnoses made solely by metagenomic NGS had a likely clinical effect, with 7 of 13 guiding treatment.

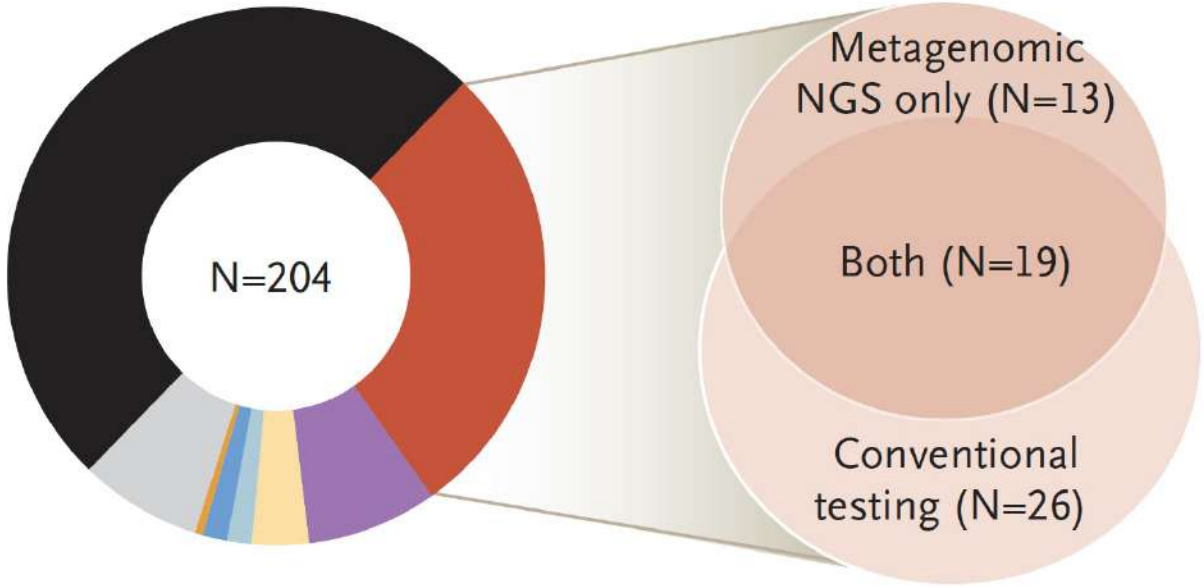
CONCLUSIONS

Routine microbiologic testing is often insufficient to detect all neuroinvasive pathogens. In this study, metagenomic NGS of CSF obtained from patients with meningitis or encephalitis improved diagnosis of neurologic infections and provided actionable information in some cases. (Funded by the National Institutes of Health and others; PDAID ClinicalTrials.gov number, NCT02910037.)

C Protocol for Metagenomic NGS Assay



A Established Diagnoses in the Study Patients

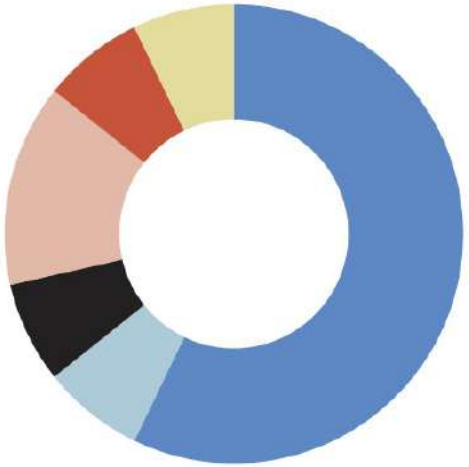


- 57 (27.9%) Infectious
- 17 (8.3%) Autoimmune
- 7 (3.4%) Neoplastic
- 3 (1.5%) Postinfectious
- 3 (1.5%) Toxic metabolic
- 1 (0.5%) Vascular
- 15 (7.4%) Other
- 101 (49.5%) Unknown

Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis

N Engl J Med 2019;380:2327-40.
DOI: 10.1056/NEJMoa1803396

E Clinical Effect (13 cases diagnosed by metagenomic NGS only)



- 7 (54%) Enabled appropriate and targeted treatment
  - *N. farcinica* — long-term treatment with oral moxifloxacin and minocycline
  - *Candida tropicalis* — treatment with high-dose fluconazole and liposomal amphotericin B (started empirically for elevated 1,3-β-D-glucan level)
  - HEV — successful treatment with IV ribavirin after patient was readmitted with liver failure and consideration of liver transplantation
  - *E. aerogenes* — narrowing of antibiotic therapy to IV cefepime and oral trimethoprim–sulfamethoxazole
  - *Enterococcus faecalis* — narrowing of antibiotic therapy to IV vancomycin; discontinuation of meropenem
  - *S. mitis* — narrowing of antibiotic therapy to IV cefepime; continuation of antibiotics for 4 wk to treat CNS infection
  - *S. agalactiae* — treatment with an additional 4 wk of therapy with IV ceftriaxone and vancomycin
- 1 (8%) Helped to rule out coinfections; enabled patient to proceed with chemotherapy (EBV-associated lymphoma)
- 1 (8%) Supported clinical decisions to narrow coverage (neisseria)
- 2 (15%) Had no effect, because patient already discharged from hospital (enterovirus)
- 1 (8%) Had no effect, because clinical significance unclear (MW polyomavirus)
- 1 (8%) Provided reassurance to patient or surrogate (SLEV)

Mikrobiyolojik Tanıda Sendromik Paneller Ülkemiz Koşullarında Ne Kadar Yararlı? Santral Sinir Sistemi (SSS) İnfeksiyonları

- SSS infeksiyonlarının tanısında sendromik panelin **yeri ayrı**
  - Bu infeksiyonlarda **doğru tanıyı en kısa sürede** koymak mortalite ve morbiditeyi önlemek açısından çok önemli
  - Panel yardımı ile **gereksiz antimikrobiyal kullanımını azaltmak, hastanede kalış süresi kısaltmak faydalı** ama daha az önemli
  - Geleneksel standart yöntemlerle **birlikte** kullanılmalı

- Ülkemiz koşullarında kullanımı, yararı?
  - **Yararlı, kullanılmalı**
  - Merkezi referans labda sunulabilir ama geri dönüş hızını (turnaround time) azaltmamalı

22. TÜRK KLİNİK MİKROBİYOLOJİ VE İNFEKSİYON HASTALIKLARI KONGRESİ

KLİMİK 2022

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Santral Sinir Sistemi İnfeksiyonları

İLGİNİZ İÇİN TEŞEKKÜRLER!

DOÇ. DR. ULUHAN SİLİ

MARMARA ÜNİVERSİTESİ TIP FAKÜLTESİ

İNFEKSİYON HASTALIKLARI VE KLİNİK MİKROBİYOLOJİ AD

uluhan@hotmail.com

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