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Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

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Optical Biosensors for Medical Diagnostics

Content

Content: Areas, where optical biosensors can provide attractive solutions:

- Areas requiring ultrasensitive detection: cancer, sepsis, and infectious diseases
- Continuous monitoring for therapeutic drug administration.
- Personalized / precision medicine

Date: June 7th

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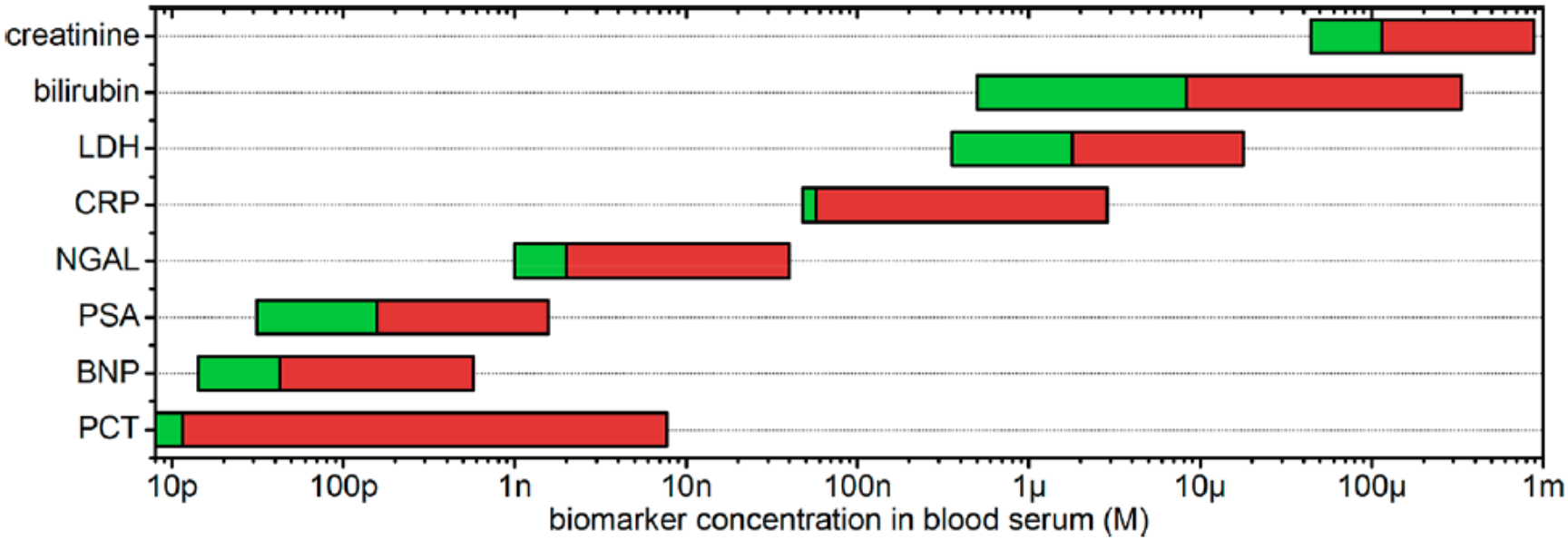
Concentration of Analytes in Clinical Samples



Typical concentration range of clinically relevant biomarkers in blood serum. The green bars indicate reference values for healthy persons, whereas the red extension to the right indicates elevated values associated with disease. LDH: lactate dehydrogenase, CRP: c-reactive protein, NGAL: neutrophil gelatinase-associated lipocalin, PSA: prostate specific antigen, BNP: B-type natriuretic peptide, PCT: pro-calcitonin

Mayo Medical Laboratories; www.mayomedicallaboratories.com, accessed 19-5-2017.

Features Biosensors Aim to Deliver / Buzzwords

Ultrasensitive: Analysis of minute amounts of target molecules

Point-of-care: Portability / simplicity for using outside clinical laboratories (at the bed site, clinician's facility, home…

Rapid analysis: timely results (minutes) allows for making decisions (e.g. cardiac markers, cytokines).

Multiplexed analysis: more complete picture enable (e.g. SARS-Cov-2 antigen and antibody detection)

Resource limited regions: solutions for countries lacking standard network of clinical labs

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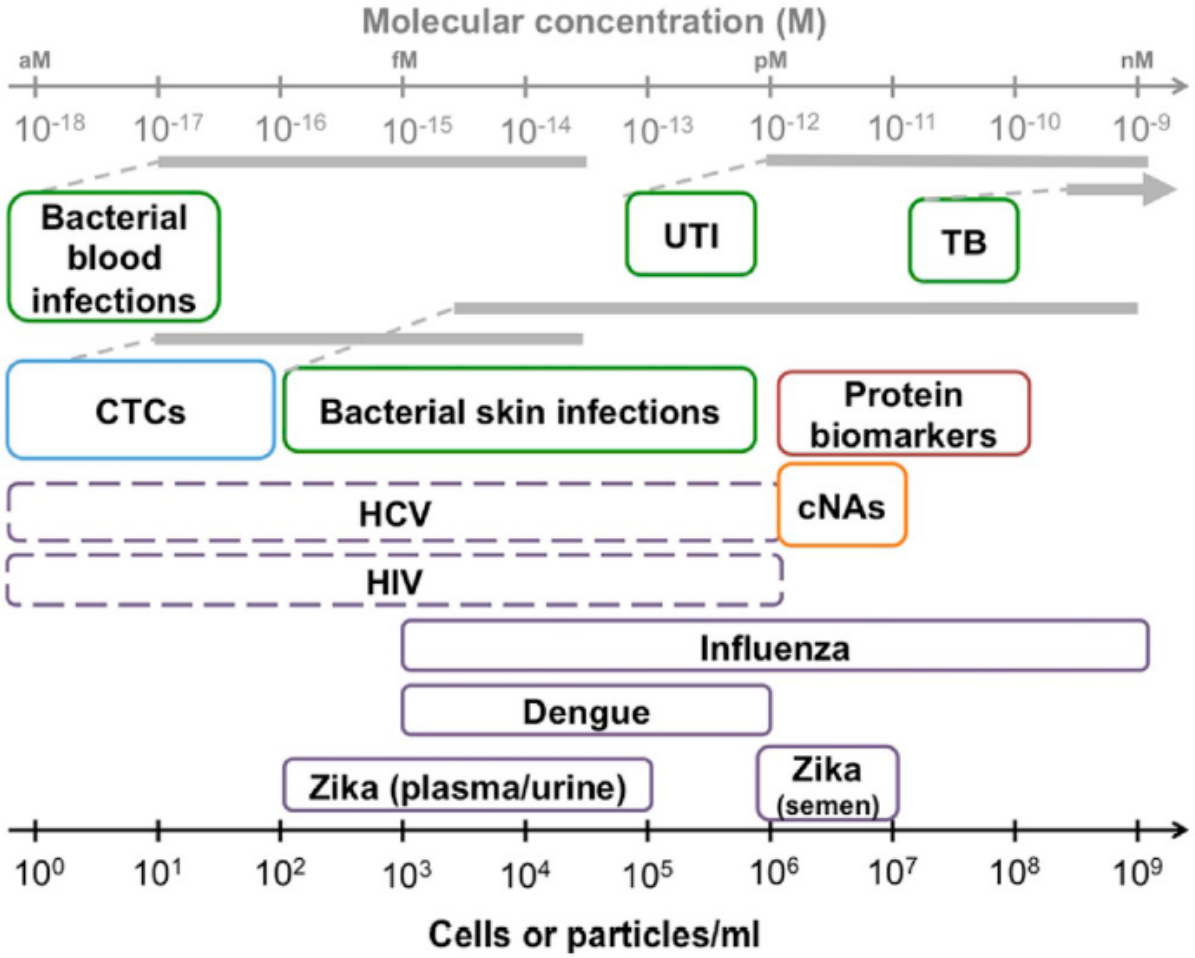
Infectious Diseases

Concentration of Viral and Bacterial Pathogens in Clinical Samples



Kelley, S.O., 2017. What are clinically relevant levels of cellular and biomolecular analytes? ACS Sens. 2 (2), 193–197.

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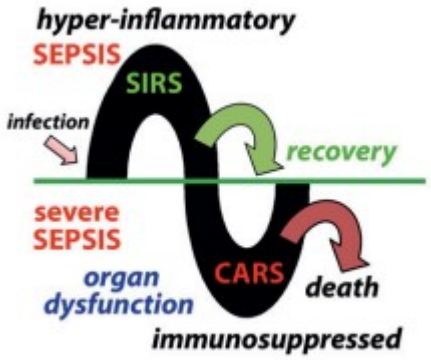
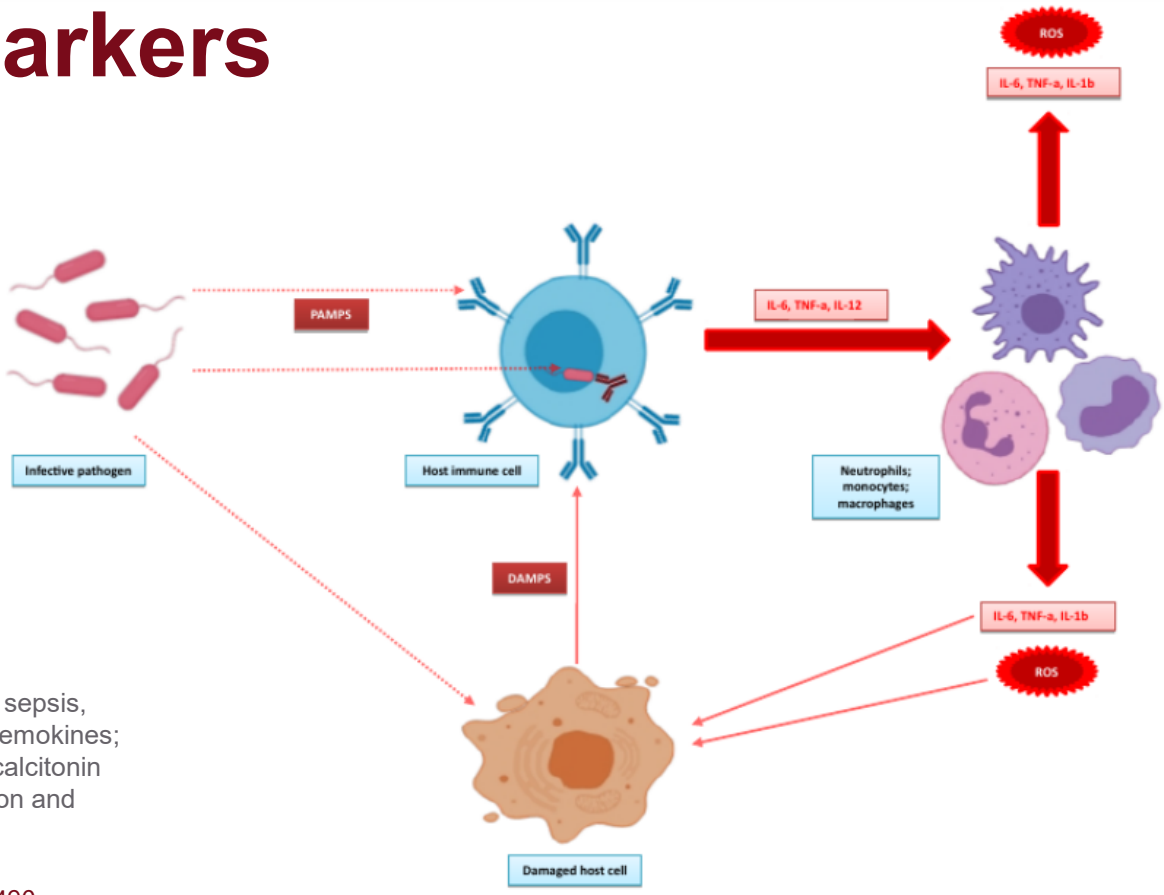
Sepsis Biomarkers





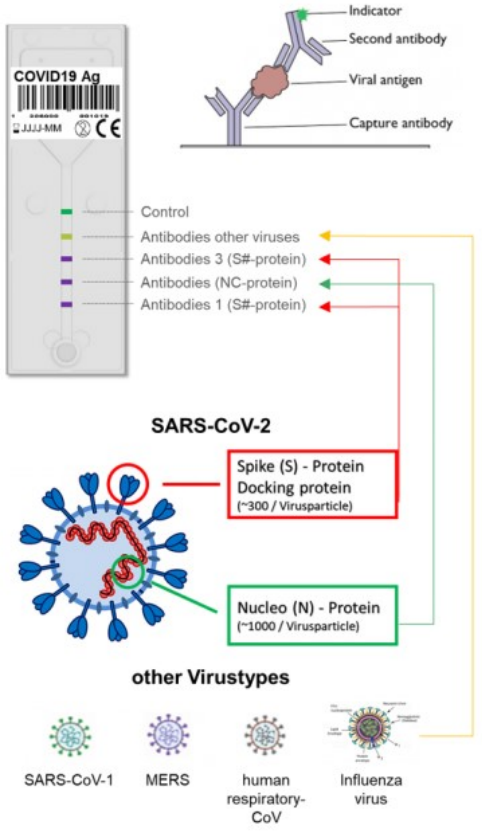
Markers of the hyper-inflammatory phase of sepsis, such as pro-inflammatory **cytokines** and chemokines; proteins such as C-reactive protein and procalcitonin which are synthesized in response to infection and inflammation.

https://doi.org/10.3109/10408363.2013.764490

Micromachines 2020, 11, 286; doi:10.3390/mi11030286

Example: Interleukin-6,8… TNF-Alpha, normal concentration <pg/mL (fM), upon sepsis can raise up to ng/mL

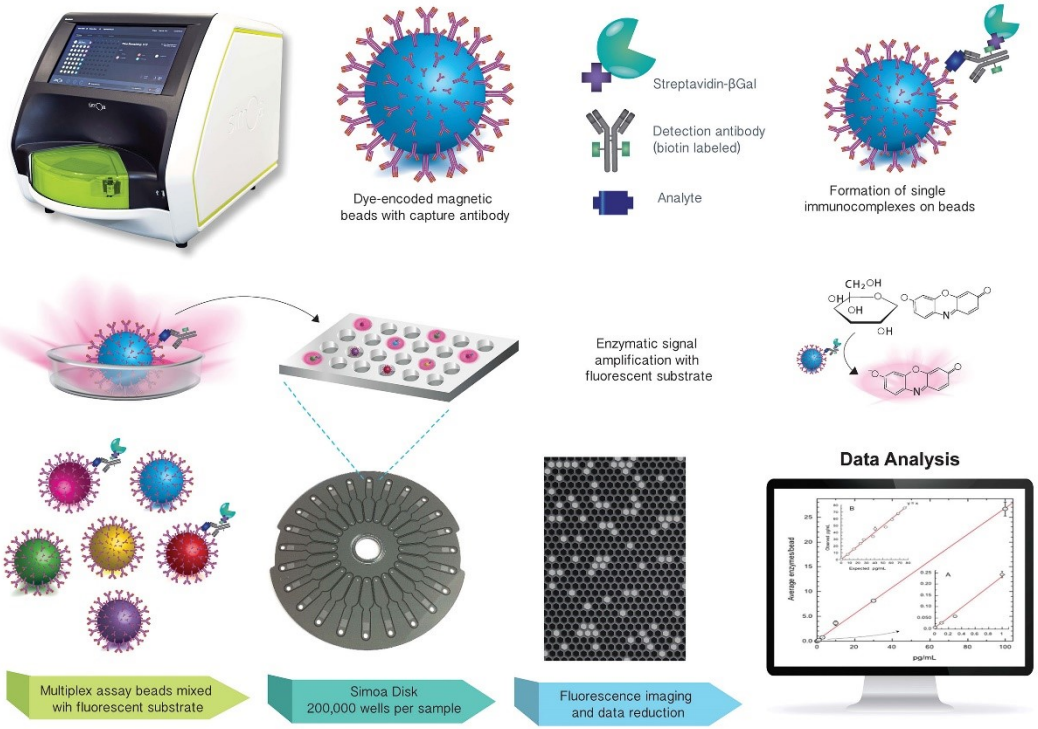
Example – Commercial SARS-Cov2 Biosensor



Chemiluminiscence – based immunoasays in a microfluidic cartridge for multiplexed (several assays) and rapid (15 min) automatized detection.

https://www.genspeed-biotech.com/

Example – Commercial SARS-Cov2 Biosensor

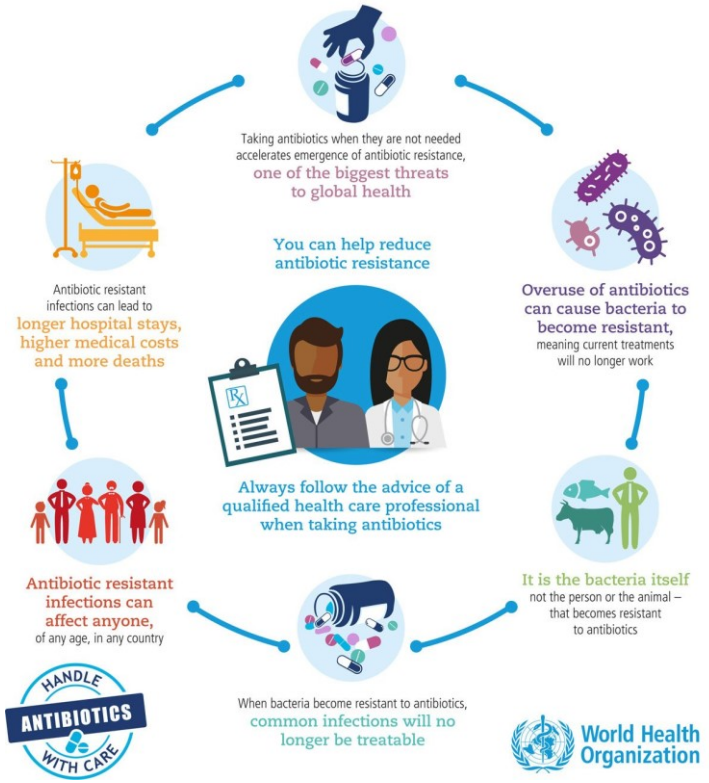


Fluorescence – based immunoasays with digital readout, used for cytokine monitoring in the Covid-19 diagnosis context

https://www.quanterix.com/

Antibiotic Resistance

- Increasing problem associated with raising number of antibiotic-resistant strands and lack of development of new antibiotics.
- Problem accelerated by the overuse of antibiotics.
- Screening of bacterial pathogen strands to choose the right antibiotic treatment can (in part) solve the problem (e.g. OXA-48 strains resistant to ampicillin…).

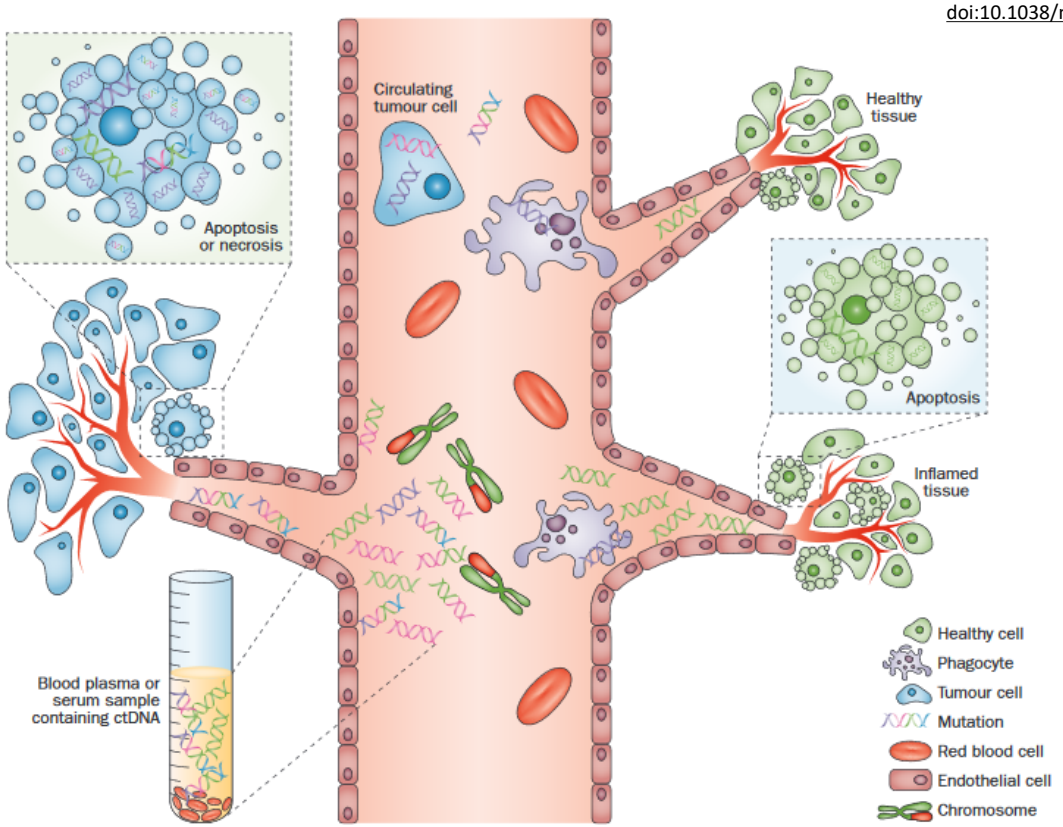


https://www.un.org/sustainabledevelopment/blog/2018/01/un-health-agency-finds-high-levels-antibiotic-resistance-worlds-common-infections/

Cancer

Liquid Biopsy

doi:10.1038/nrclinonc.2013.110



- Specific detection of tumor-derived cell free DNA (cfDNA) and other markers leaching to blood (rather then analyzing invasively collected tumor tissue).

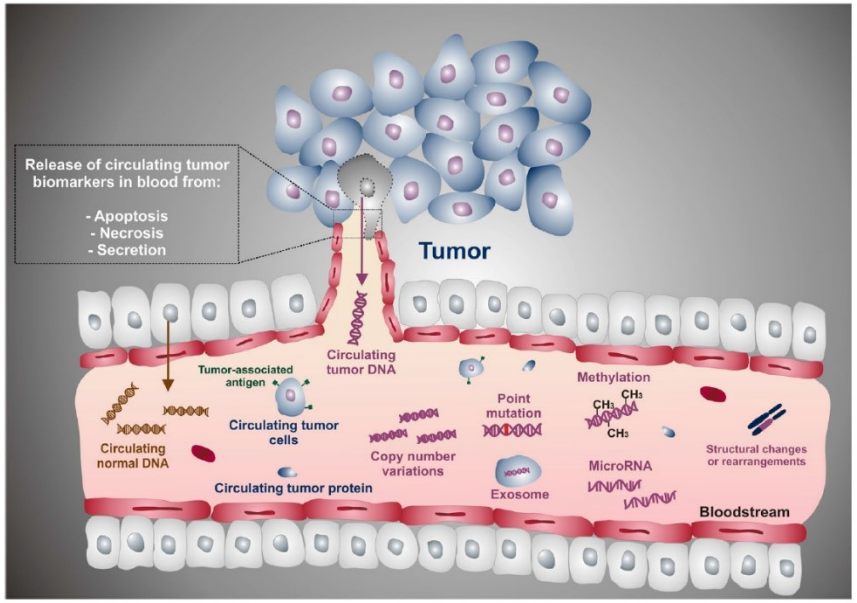
Liquid Biopsy

Table 1 | Tumour-associated genetic aberrations in circulating free DNA

Tumour type	Stage	*n*	Tumour-specific aberration	Tumour burden or stage*	Source	Technique	Reference‡
Colorectal cancer	Early to advanced	33	*APC*	No/Yes	Plasma	BEAMing	Diehl *et al.* (2005)[36]
	Advanced	18	*APC, KRAS, PIK3CA, TP53*	Yes	Plasma	BEAMing	Diehl *et al.* (2008)[27]
	Early to advanced	104	*APC, KRAS, TP53*	NA	Serum	PCR-SSCP	Wang *et al.* (2004)[28]
	Early to advanced	70	*KRAS*	NA	Plasma	ME-PCR	Frattini *et al.* (2008)[34]
Breast cancer	Early to advanced	72	*PIK3CA*	Yes	Plasma and serum	ARMS-Scorpion PCR	Board *et al.* (2010)[40]
	Early to advanced	34 (retrospective) and 51 (prospective)	*PIK3CA*	NA	Plasma	BEAMing	Higgins *et al.* (2012)[42]
	Advanced	30	*PIK3CA, TP53,* structural variation	Yes	Plasma	TAm-Seq and digital PCR	Dawson *et al.* (2013)[29]
Ovarian cancer	Advanced	38	*TP53, PTEN, EGFR, BRAF, KRAS,*	Yes	Plasma	TAm-Seq Digital PCR	Forshew *et al.* (2012)[38]
	Early to advanced	63	*PIK3CA*	Yes	Serum	Fluorescent-PCR	Kuhlmann *et al.* (2012)[52]
Hepatocellular carcinoma	Early	4	SNV	Yes	Plasma	WGS	Chan *et al.* (2013)[9]
Pancreatic cancer	Early to advanced	21	*KRAS*	Yes	Plasma	MASA PCR	Yamada *et al.* (1998)[54]
	Early to advanced	44	*KRAS*	No/Yes	Plasma	RFLP-PCR	Castells *et al.* (1999)[53]
Oral squamous-cell carcinoma	Early to advanced	64	Microsatellite loci	Yes	Serum	PCR	Hamana *et al.* (2005)[50]
	Early to advanced	20	Microsatellite loci	No	Serum	PCR	Kakimoto *et al.* (2008)[157]
Non-small-cell lung cancer	Advanced	246	*KRAS*	Yes	Plasma	ARMS-qPCR	Nygaard *et al.* (2013)[59]
Breast and osteosarcoma	Advanced	3	Genomic alterations	Yes	Plasma and serum	Nested-real time PCR	McBride *et al.* (2010)[154]
Colorectal and breast cancer	Advanced	10	Chromosomal alterations	Yes	Plasma	WGS	Leary *et al.* (2012)[37]

*This column indicates if the study observed a correlation between tumour-associated genetic aberrations and tumour burden or disease stage. ‡The table includes studies in which different tumour-associated genetic aberrations have been detected using a variety of techniques, with different cancer types and at different stages. Abbreviations: ARMS, amplification refractory mutation system; BEAMing, beads, emulsion, amplification, magnetics; MASA, mutant allele specific amplification; ME-PCR, mutant enriched PCR; NA, not applicable; PCR-SSCP, single-strand conformation polymorphism PCR; qPCR, quantitative PCR; RFLP-PCR, restriction fragment length polymorphism PCR; SNV, single nucleotide variants; WGS, whole-genome sequencing.

Cancer Biomarkers



Bellassai, N; Spoto, G, (18 July 2016). "Biosensors for liquid biopsy." (Anal Bioanal Chem) doi: 10.1007/s00216-016-9806-3

Genetic and epigenetic markers

- mostly focused on tumor DNA
- e.g. KRAS, TP53, APC
  - Cologuard stool test
  - ColoVantage Plasma blood test

Protein markers

- Tumor Associated Antigens (TAAs)
- **Antibodies against TAAs**
- Other CRC related proteins

- *Early cancer diagnosis*, prognosis, patient follow-up and therapy efficacy - requires sensitivity (and not necessarily speed).
- Cancer is a very complex disease and typically cannot be diagnosed by detecting individual marker – requires multiplexing.

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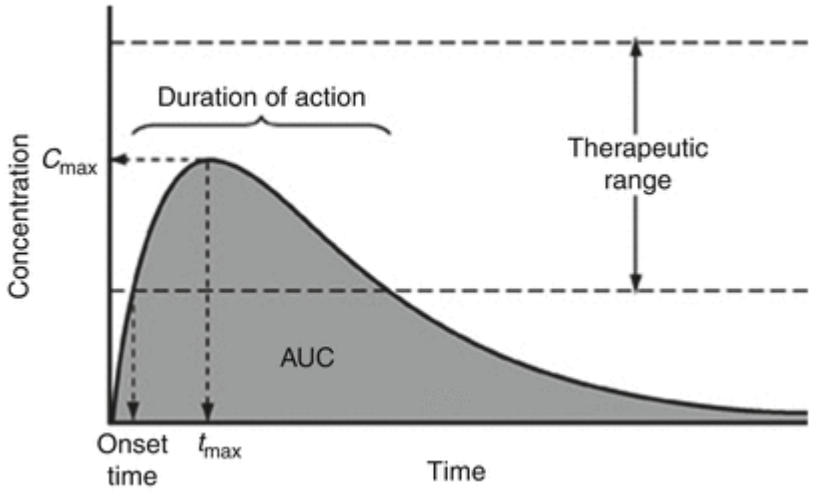
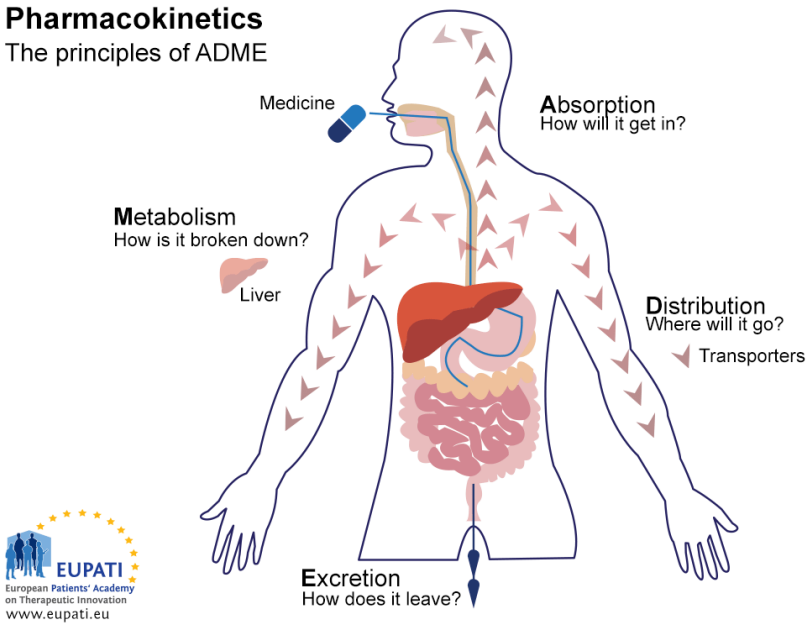
Therapeutic Drug Administration

Pharmacokinetics

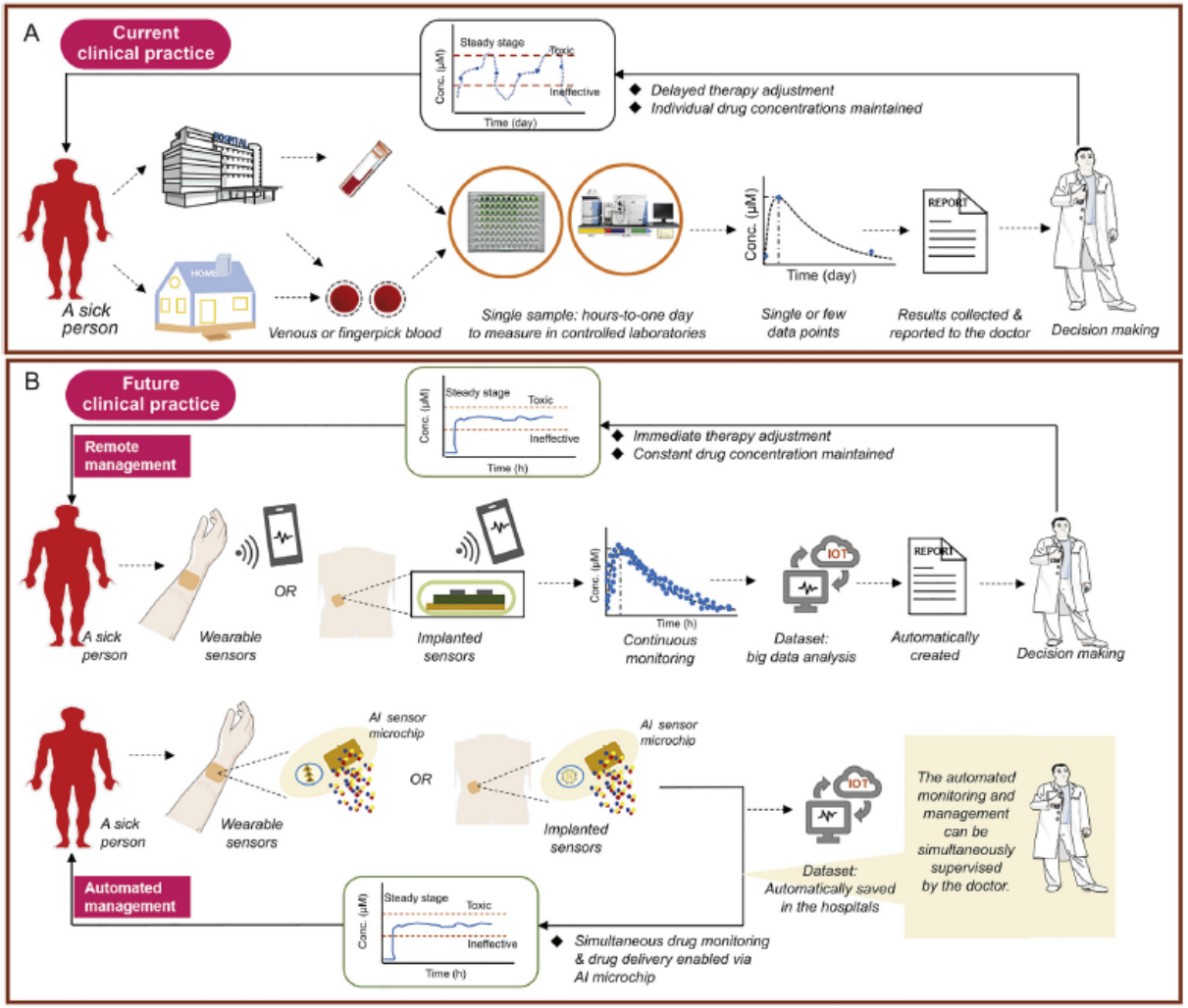




https://toolbox.eupati.eu/glossary/pharmacokinetics/

- The dosage of drug should assure concentration below the toxic range and above the threshold.
- Certain drugs exhibit very narrow therapeutic range such as by methotrexate (chemotherapy), theophylline (asthma)…

Continuous Monitoring of Therapeutic Drugs



https://doi.org/10.1016/j.jpha.2020.08.001

Continuous Monitoring of Therapeutic Drugs

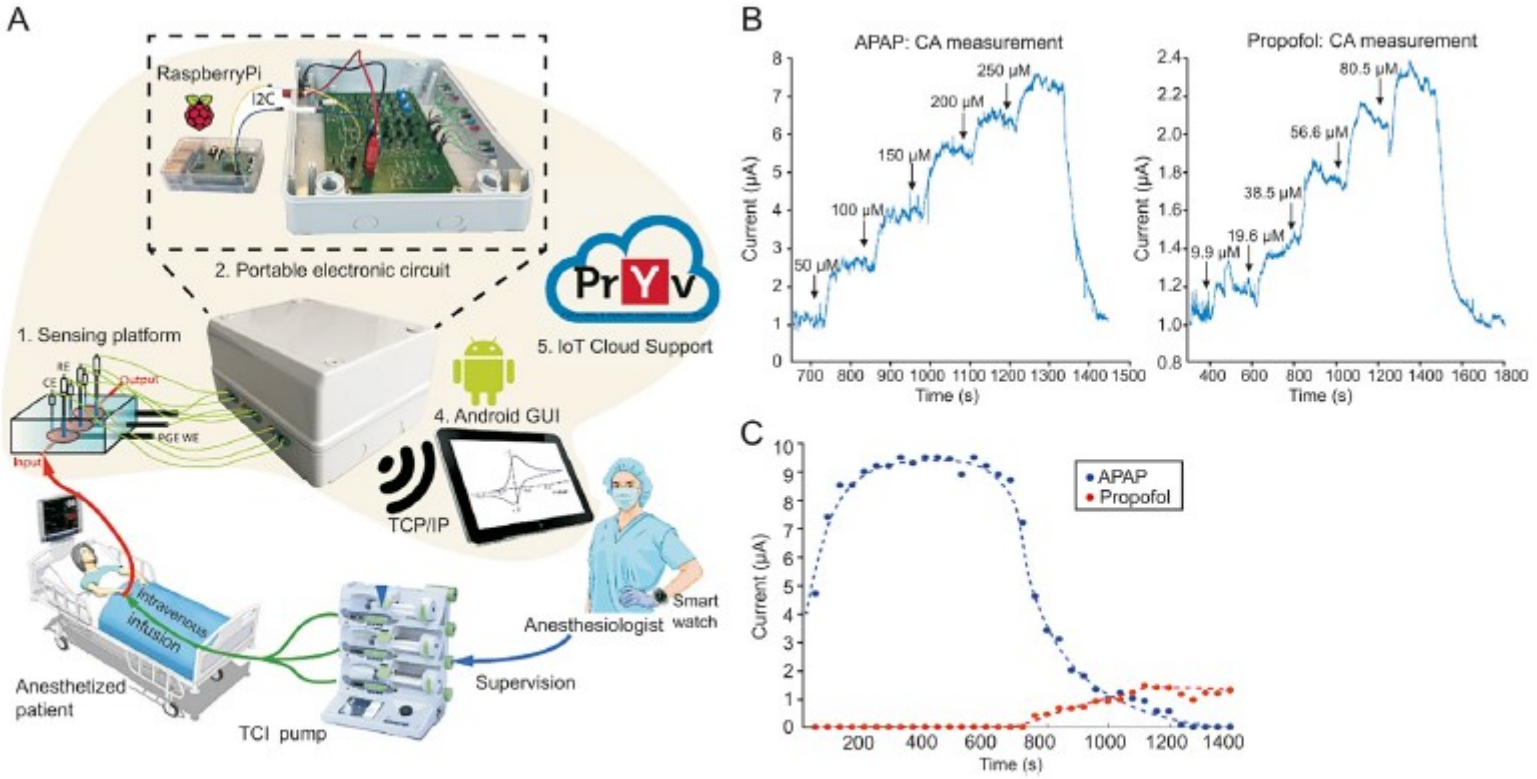
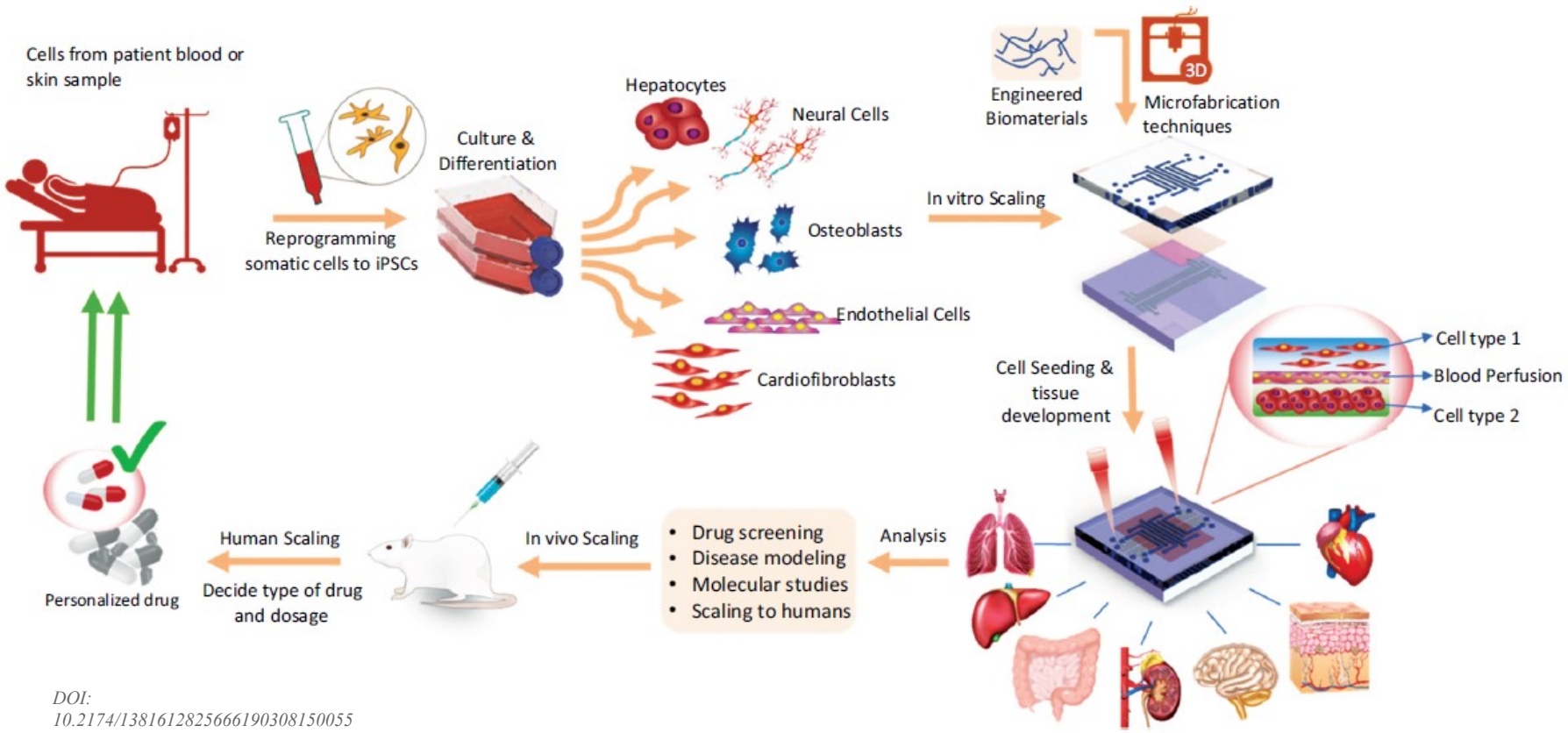


Fig. 2. Schematic representation of an electrochemical sensor for in vitro continuous drug monitoring (CDM). (A) An IoT system for continuous and simultaneous online monitoring of two anaesthetics: paracetamol (APAP) and propofol, proposed for integration into clinical practice; (B) chronoamperometry measurements of APAP and propofol; (C) real-time monitoring of the two drugs in undiluted human serum for over 24 min [37]. TCP/IP: Transmission Control Protocol/Internet Protocol; GUI: graphical user interface; TCI: target controlled infusion; CA: chronoamperometry.

F. Stradolini, A. Tuoheti, T. Kilic, et al., An IoT solution for online monitoring of anesthetics in human serum based on an integrated fluidic bioelectronic system, IEEE Trans. Biomed. Circuits Syst. 12 (2018) 1056e1064.

Personalized / Precision Medicine



DOI:
10.2174/138161282566190308150055

- Organ-on-a-chip (OOC) platforms are pursued for personalized drug discovery process
- Combination with sensing on the chip may provide means for analysis of response to the investigated therapeutic drugs and their cocktails.

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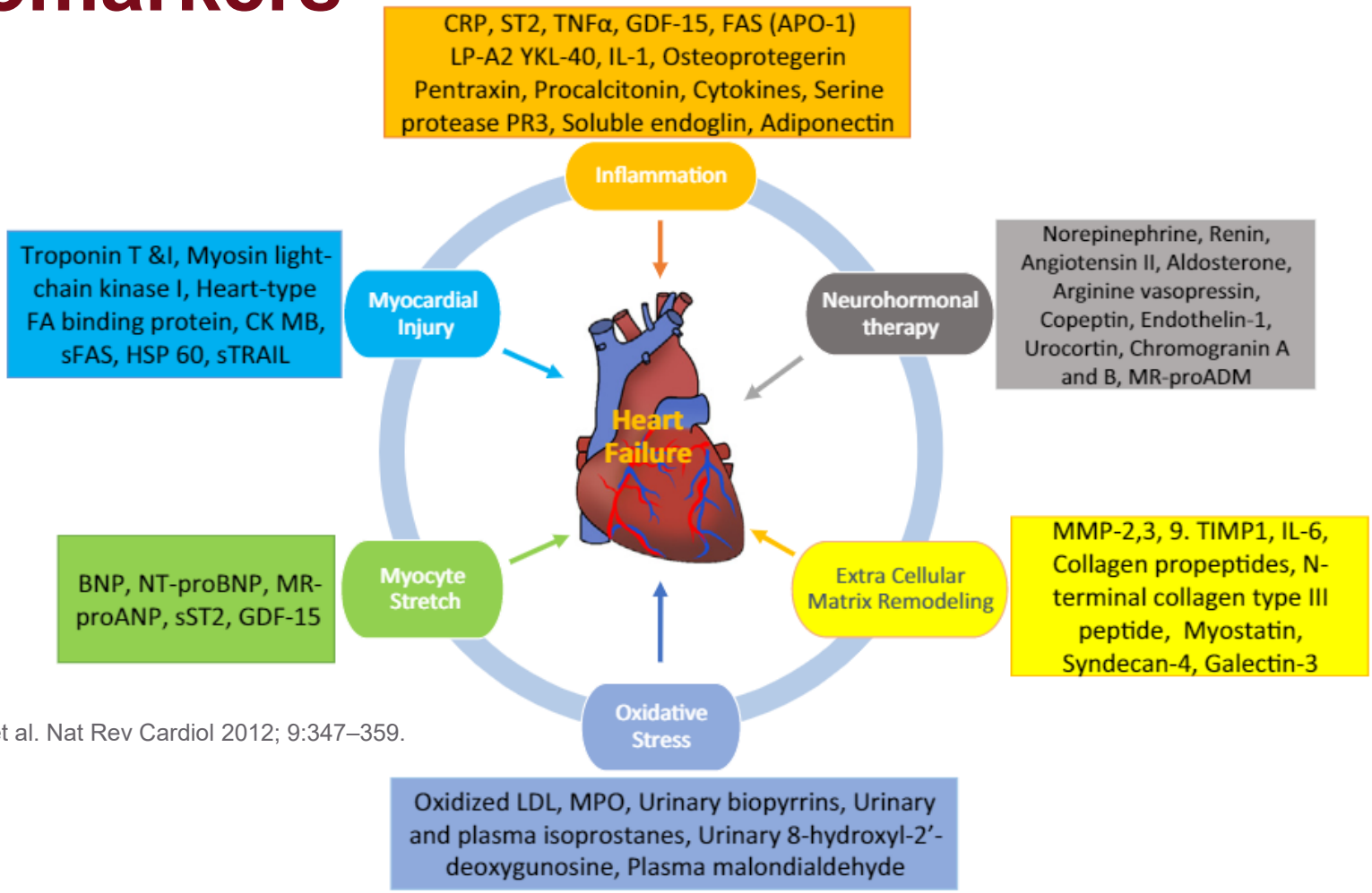
Rapid Diagnosis

Cardiovascular Diseases Biomarkers



Ahmad et al. Nat Rev Cardiol 2012; 9:347–359.

- Classification of heart failure biomarkers according to pathophysiologic processes.

Troponin

- The troponin complex consists of three subunits: troponin T (cTnT), troponin I (cTnI) and troponin C (cTnC).
- cTnI is confined inside the heart muscle and it is standard biomarker for acute myocardial infraction (AMI). Early troponin I detection would lead to faster diagnosis and consequently the initiation of the correct treatment
- cTnI levels begin to rise 2–3 h after the myocardial infarction and elevation of its levels can persist for up to 10 days, making it ideal for retrospective diagnosis of infarctions.
- It has been demonstrated that testing troponins on patient admission and again after 6–12 h provides better risk stratification and early diagnosis.
- The borderline between normal people and patients is 20 pM to 83 pM cTnI concentration
- While after the outbreak of AMI, this concentration can go up to 2 nM within 3–6 h, and levels at about 20 nM for 6–8 days.

Anil Bozdogan, Reham Kased, Vanessa Jungbluth, Wolfgang Knoll, Jakub Dostalek , Amal Kasry, Development of a Specific Troponin I Detection System with Enhanced Immune Sensitivity using a Single Monoclonal Antibody, 2020, Royal Society Open Science, 7: 200871