LEARNING THE ROPES:

Angiotensin Metabolites in the diagnostic workup of hypertension

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More than 50% of patients on antihypertensive therapy remain hypertensive. Identifying the individual cause for uncontrolled hypertension remains a major challenge for physicians. Underdosing, lack of treatment adherence, whitecoat and resistant hypertension but also secondary forms of hypertension are among the most common causes of uncontrolled hypertension. Identification of these subtypes and therefore enabling individualized therapeutic approaches is key to improve hypertension control. Simple and reliable diagnostic tools for molecular profiling of hypertension are desperately needed to bring light into the black box of uncontrolled hypertension.

The renin angiotensin aldosterone system (RAAS) is the central pharmacologic target in the treatment of hypertension. Angiotensin-converting-enzyme (ACE) inhibitors (ACEi) and angiotensin receptor blockers (ARBs) are part of every first-line therapy recommended by clinical guidelines¹. The key effector hormone of RAAS is angiotensin II (Ang II). Ang II binds to Ang II type-1 receptors (AT1R) resulting in direct vasoconstriction. In adrenals, AT1R stimulation by Ang II results in upregulation of aldosterone synthesis and secretion. The RAAS is a humoral peptide hormone cascade that is abundantly fueled with the pro-hormone angiotensinogen (AGT) secreted by the liver. AGT is cleaved by renin, the secretion of which is tightly regulated in the kidneys. Renin mediated cleavage of AGT results in the release of angiotensin I (Ang I). Endothelial and circulating ACE convert Ang I to Ang II, which then can act through its vascular and adrenal receptors. With its key functions within the RAAS, either blocking Ang II formation or signaling became key strategies in the pharmacologic treatment of hypertension using ACEi and ARBs.

Molecular diagnostics in hypertension is currently limited to the measurement of renin and aldosterone

using different technologies. Of note, antihypertensive medications strongly interfere with these readouts², which can be problematic when interpreting test results. Consequently, the use of aldosterone and renin, especially in the context of using the aldosterone-to-renin ratio (ARR) in screening for primary aldosteronism (PA) requires drug withdrawal to maintain screening sensitivity, which is a major barrier for broad clinical implementation of PA screening. Moreover, the lack of certified reference standards for active renin as well as methodological caveats of antibody-based methods for detection of renin and aldosterone³ result in poor comparability of cut-off values between different methods and even clinical centers, resulting in low screening rates and severe underdiagnosis of PA. In PA, adrenal aldosterone secretion becomes independent of Ang II. The underlying pathology involves aldosterone producing cell clusters (APCCs), bilateral adrenal hyperplasia and aldosterone producing adenomas, which in total are thought to affect 5-10% of all hypertensive patients. In PA, the aldosterone excess leads to sodium and water retention and a subsequent volume increase culminating in angiotensin independent hypertension. Consequently, first-line therapies using ACEi and ARBs are inefficient in treating PA, as the molecular cause for hypertension is downstream of ACE and AT1R. ACEi and ARBs strongly interfere with the current screening strategy for PA, which leads to the requirement of drug withdrawal before PA screening using the ARR². Although renin concentration is widely used as a surrogate marker for RAAS activation in PA screening, the effector hormone Ang II is only partially reflected by the renin concentration. Patient specific variations in ACE activity, especially in the presence of ACEi therapy result in a loss of the correlation between renin concentration and Ang II levels, which is one of the reasons why screening for PA is prone to drug interference artifacts if using renin based diagnostic ratios like the ARR. Compensatory up-regulation of renin results in a suppression of the ARR,

which is used as a screening test for PA. Recently, Ang II has been introduced as an alternative denominator to be used in PA screening⁴. By using Ang II instead of the surrogate marker renin as a measure for RAAS activation, the direct link between the Ang II and adrenal aldosterone secretion seems to provide a more solid diagnostic ratio for PA screening. The Aldosterone-to-Ang II-ratio (AA2-Ratio) remained elevated while the ARR was significantly suppressed in PA patients treated with ACE inhibitors⁵.

With the availability of LC-MS/MS based RAAS equilibrium analysis, angiotensin levels became available for applications in clinical routine. In contrast to immunoassay-based approaches, LC-MS/MS is a direct quantification method allowing for easy internal standardization and analyte multiplexing, which reduces costs and increases accuracy of the test results. Recently, the RAAS Triple-A test has been introduced for molecular hypertension profiling. The assay simultaneously determines Ang I, Ang II and aldosterone in serum samples and is available as an LC-MS/MS kit (Attoquant Diagnostics, Vienna), that is compatible with clinical LC-MS/MS systems. Obtained angiotensin levels are used to calculate angiotensin-based markers for renin activity (PRA-S), ACE activity (ACE-S) and adrenal AT1R function (AA2-Ratio). Measured analytes and derived enzyme markers are obtained from a single analytic run and are interpreted in the context of the existing drug prescription. Both, ACEi and ARBs induce specific changes in angiotensin profiles and derived biomarkers,

which can be used to evaluate the pharmacologic efficacy of RAAS blockers in clinical samples. In contrast to measuring drug molecules in serum during therapeutic drug monitoring, RAAS Triple-A parameters are used to evaluate the physiological responses induced by antihypertensive drugs. Therefore, drug efficacy monitoring using angiotensin levels remains independent of the type of drug molecule. The approach is currently being validated in a large population-based cohort and for the first time offers drug efficacy monitoring and screening for secondary hypertension in a single diagnostic approach.

In summary, the utilization of angiotensin metabolites in hypertension profiling moves clinical diagnostic approaches away from surrogate markers and introduces the actual effector hormones of the RAAS into clinical diagnostics. Selected angiotensin metabolite ratios can be utilized as markers for pharmacologic efficacy of ACE inhibitors and ARBs while simultaneously screening for secondary forms of hypertension. With the broad availability of the RAAS Triple-A test for clinical mass spectrometry laboratories, multiple causes of uncontrolled hypertension can be simultaneously addressed in a single diagnostic workup that only requires a simple blood collection on standard antihypertensive therapy. The interpretation of a patient's angiotensin metabolite levels and derived biomarkers in the context of the existing drug prescription could be a gamechanger in hypertension management, allowing for individualized and effective therapeutic approaches.

Figure 1: Schematic representation of the RAAS.

REFERENCES

1. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, Ramirez A, Schlaich M, Stergiou GS, Tomaszewski M, Wainford RD, Williams B, Schutte AE. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. *Hypertension*. 2020 Jun;75(6):1334-1357. doi: 10.1161/HYPERTENSIONAHA.120.15026. Epub 2020 May 6. PMID: 32370572.
2. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M, Young WF Jr. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2016 May;101(5):1889-916. doi: 10.1210/jc.2015-4061. Epub 2016 Mar 2. PMID: 26934393.
3. Guo Z, Poglitsch M, McWhinney BC, Ungerer JPJ, Ahmed AH, Gordon RD, Wolley M, Stowasser M. Aldosterone LC-MS/MS Assay-Specific Threshold Values in Screening and Confirmatory Testing for Primary Aldosteronism. *J Clin Endocrinol Metab*. 2018 Nov 1;103(11):3965-3973. doi: 10.1210/jc.2018-01041. Erratum in: *J Clin Endocrinol Metab*. 2019 Jun 1;104(6):2054-2056. PMID: 30137438.
4. Burrello J, Buffolo F, Domenig O, Tetti M, Pecori A, Monticone S, Poglitsch M, Mulatero P. Renin-Angiotensin-Aldosterone System Triple-A Analysis for the Screening of Primary Aldosteronism. *Hypertension*. 2020 Jan;75(1):163-172. doi: 10.1161/HYPERTENSIONAHA.119.13772. Epub 2019 Nov 25. PMID: 31760886.
5. Guo Z, Poglitsch M, Cowley D, Domenig O, McWhinney BC, Ungerer JPJ, Wolley M, Stowasser M. Effects of Ramipril on the Aldosterone/Renin Ratio and the Aldosterone/Angiotensin II Ratio in Patients With Primary Aldosteronism. *Hypertension*. 2020 Aug;76(2):488-496. doi: 10.1161/HYPERTENSIONAHA.120.14871. Epub 2020 Jun 8. PMID: 32507039.

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