A comparison of PCR methods for the detection of MYD88 L265P mutations in a diagnostic setting

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Introduction

Waldenström’s Macroglobulinemia (WM) is a B-cell disorder (BCD) presenting with lymphoplasmacytic cell infiltration of the bone marrow (BM) and a clonal IgM (immunoglobulin-M) secretion. Diagnosis of WM is problematic as characteristics overlap with other BCDs. MYD88 L265P is found in 91% WM cases. MYD88 L265P was found to a lower extent in other BCDs (3% CLL and 29% ABC-DLBCL).

MYD88 is part of a signalling complex, resulting in prosurvival signalling caused by translocation of NF-kB to the nucleus (Figure 1).

Xu et al (2013) developed an AS-PCR (conventional allele specific PCR) and RT-PCR (real-time PCR) method to detect MYD88 L265P mutations.

CXCR4 mutations are the second most common mutation in WM. Almost all WM patients with CXCR4 mutations exhibit MYD88 L265P. CXCR4 mutations amplify the effects of WM by prolonging signalling.

Aims

• Compare the AS-PCR and RT-PCR method of MYD88 L265P detection to determine which method is best in a diagnostic setting.
• Identify the incidence of MYD88 L265P in a range of BCDs from KCH
• Identify the CXCR4 status of MYD88 L265P patients from KCH
• AS-PCR
• RT-PCR

Methods

AS-PCR

RT-PCR

Results

MYD88 L265P is detectable by AS-PCR

AS-PCR was able to detect MYD88 L265P in serially diluted DNA from 13% to 0.625% at 5ng/μl (Figure 2).

AS-PCR is subject to factors affecting MYD88 L265P detection

Visualisation of a band is difficult when amplification is suboptimal due to high DNA concentration. Low level MYD88 L265P burden (0.625%) produces inconsistent amplification. The AS-PCR assay is also prone to operator error (Figure 3).

MYD88 L265P is detectable by RT-PCR

RT-PCR reliably detects MYD88 L265P in diluted DNA from 13% to 0.625% at 5ng/μl (Table 1, Figure 4 & 5).

The RT-PCR method reliably detects MYD88 L265P at 0.625% with DNA at 5ng/μl. The AS-PCR method is able to detect MYD88 L265P DNA at 0.625% at 15ng/μl but not at 5ng/μl (Figure 6).

The RT-PCR method reliably detects MYD88 L265P at 0.625% with DNA at 5ng/μl. The AS-PCR method is able to detect MYD88 L265P DNA at 0.625% at 15ng/μl but not at 5ng/μl (Figure 6).

Therefore it is clear that the RT-PCR is a more reliable and sensitive method of detecting MYD88 L265P.

Discussion

The AS-PCR assay is subject to factors affecting MYD88 L265P detection particularly at low levels of MYD88 L265P whereas RT-PCR is not affected and detects MYD88 L265P with lower DNA concentrations. Low tumour burden is common amongst WM therefore high sensitivity is vital.

Frequency of MYD88 L265P was comparable to established literature in WM, however in IgM-MGUS and MZL the MYD88 L265P levels were lower and higher respectively, however this may be due to smaller sample size.

Targeting components of the signalling pathway MYD88 is involved in including NF-κB and BTK results in decreased WM cell survival.

Treatment of WM with Brutinib improved progression-free survival, however CXCR4 mutations promote resistance to Brutinib. Therefore identifying the MYD88 and CXCR4 status is important with regards to effective treatment.

Conclusion

• The RT-PCR assay for MYD88 L265P detection is preferred to the AS-PCR assay
• The MYD88 L265P detection rate in WM is comparable to established literature
• CXCR4 mutations were detectable in MYD88 L265P patients by next generation sequencing

References

Xu, L., Hunter, Z.R., Yang, G., Zhou, Y., Cao, Y., Liu, X., Morra, E., Trojani, A., Greco, A., Arcaini, L., Varettoni, M., Br...